STUDIES ON THE LIFE HISTORY AND ECOLOGY OF *CUTEREBRA* SPP. OCCURRING IN MICHIGAN COTTONTAILS WITH SYSTEMATIC STUDIES ON CUTEREBRINE LARVAE FROM OTHER MAMMALS

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STUDIES ON THE LIFE HISTORY AND ECOLOGY OF *CUTEREBRA* SPP. OCCURRING IN MICHIGAN COTTONTAILS WITH SYSTEMATIC STUDIES ON CUTEREBRINE LARVAE FROM OTHER MAMMALS

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DEDICATION

The author wishes to dedicate this thesis to those dear ones, Michelle and Mary Denise, and to Mrs. Mary Boisvenue whose belief led to this accomplishment.

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INTRODUCTION AND HISTORICAL REVIEW

The purpose of this study was (1) to investigate the phases of the life history of Cuterebra sp. as they occur under natural conditions, (2) to elucidate the ecology of the cuterebrid flies in relation to vegetation, soil and host, the Michigan cottontail, Sylvilagus floridanus mearnsii, and (3) to clarify the systematics of cuterebrine larvae collected from certain mammals.

A. LIFE HISTORY

At present the biological knowledge of the Cuterebra species is rather scant. The paucity of material is noticed in the problem of the life cycle, which Cameron (1926) states may be attributed to the shy habits of the cuterebrid adult flies. Reviews by Hadwen (1915), Townsend (1917), Crawley (1923) suggest that the possible mode of infection in carnivores may be through the ingestion of rodent carcasses, having immature larvae. From the stomach these larvae migrate to the skin in the new host and there complete their development. Parker and Wells (1919) offered partial evidence in support of this idea by finding subcutaneous infestations in prairie dogs orally given young larvae. Hall (1925) states that this mode of infection might explain

the finding of a last stage cuterebrid larva in the nostrils of a cat. The cat in the process of feeding on an infected carcass may have had a "grub" entering the nostrils. Evidence of carnivores swallowing cuterebrid larvae was presented by the above author in cases of late stage larvae found in the stomach and fecal samples of carnivores.

Other contingencies mentioned in the papers of Cameron (loc. cit.), Hall (loc. cit.), Dalmat (1943) are that the infestation of the carnivores may be direct, i.e. that the adult female fly deposits its eggs on the host. Dalmat (loc. cit.) further states that the host licks the eggs off the body hairs, and swallows the infective eggs. As a modification of the above possibility, the deposited eggs hatch into first stage larvae and may penetrate the skin of the host by means of strong oral hooks, and burrow into the subcutis. The morphology of the egg reflects the possibility of a strong attachment to the hairs of the host by a glutinant ventral groove (Hadwen, loc. cit.; Parker et al., loc. cit.).

Townsend (1935) offered the probability that the eggs are laid on food plants of the host and when the animal feeds, the eggs are ingested. Once in the stomach of the host, the eggs excyst releasing the active larvae, which through some unknown migration route reach the subcutaneous region of the body.

The fact that hosts are highly infested in the throat and neck regions may be explained by their frequent brushing

against vegetation having first stage larvae (Allison, 1953).

Townsend (1915) suggested that the eggs are probably deposited in burrows or runways of the rodents. Adult cuterebrids, according to Chandler (1949), lay great quantities of eggs in areas rabbits frequent. Beamer's observation (1950) supports this evidence, in that female flies were seen laying eggs in the runways of the host.

Townsend (1935) observed that cuterebrid flies frequented rockpiles, brushpiles, and rock recesses that could be rodent habitats. Dalmat (1943) conjectured on the above observation that it seemed likely to find cuterebrine eggs deposited in nests and burrows used by hosts. Consequently, hatched first stage larvae would probably come in contact with the habitant host, attach to it, wander and burrow in when a suitable site was reached. In the subcutis they would continue developing to mature larvae. Beamer and associates (1943) supported the above conjecture by discovering eggs around the burrows of pack rats. The eggs were found randomly on logs, under stones, sticks in or over the entrance of the nests. Hall (1925) offered the possibility of prenatal infection, which is demonstrated in the case of parasites, having larvae that wander through the host tissues. That author stated further that there is no evidence of this migration as yet, in Cuterebra species. This hypothesis might clarify some questions on early infections of nursing animals cited by Dalmat (loc. cit.).

Thus from the data on the life cycle of Cuterebra sp. there appears to be two hypotheses; the direct ingestion mode, and the skin penetration mode of infection.

Observations by Dalmat (1943) on the oviposition of female cuterebrid flies showed that numerous eggs were laid ("more than 1000 eggs have been taken from one Cuterebra"). Measurements of the eggs resulted in an average length and width of 1.4 mm. and 0.3 mm. respectively. A total time of oviposition for 450 eggs was 45 hours. The female fly exhibited a sporadic egg-laying two days after it had emerged. The pattern of the deposited eggs on a twig revealed eggs singly-laid in a row. Characteristics of the eggs such as a very heavy chorion, a ventral groove, a sticky substance on the posterior end, and an operculated anterior end were recorded by the above author. These features corroborated Hadwen's findings (1915). Dalmat (loc. cit.) goes on to relate that oviposition probably does not happen on the host owing to the numerous eggs laid. Thus a high mortality rate would result because of the reduced number of first stage larvae reaching a suitable host. Parker and Wells (1919) determined the longevity of collected viable eggs to be six months.

Whether the operculum of the egg is opened by the friction and moisture in the mouth (Townsend, 1935) or if the larvae emerge from eggs without mechanical aid (Moilliet,

1943; Ferris, 1920), the first stage larvae actively leave the eggs to establish themselves in the host. Moilliet (loc. cit.) retained one unfed larva for ten days.

With the establishment of the first stage larva in the host, Knipling and Brody (1940) found that a molting period follows at the end of which a second stage larva evolves. The metamorphosis to the third stage larva is preceeded by an intermediate molting period. Mature larvae are usually defined as those found in the late third stage (Dalmat, 1942). After some time the mature larva drops from the host, burrows in the ground, and changes into a pupal stage (Ryckman, 1953). The pupa overwinters in the soil, according to Dalmat (loc. cit.) and in late spring or early summer, depending on date of pupation, an adult fly emerges from the puparium. In southern Georgia, Knipling and Brody (loc. cit.) found that two generations a year may exist.

In the investigation of the cuterebrid parasites a problem confronting workers is that of the length of larval development in the host. Reviews by Vorhies and Taylor (1940), Moilliet (1943), relate that the larval period of rodent Cuterebra species is from three to four weeks in duration. These observations compared with those of Scott and Snead (1942) in their finding of a larval infection period as approximately one month. One observation suggests that the developmental period of cuterebrid species is relatively much shorter than those of Hypoderma and Gasterophilus

(Knipling et al., 1940). These authors stated further that the duration of the second stage larvae was determined to be 4 to 5 days. This duration began at the appearance of an opening in the host's skin until the molt to the third stage. The third or mature stage required 10 days. No specific data could be found on the time of the first stage larvae to establish itself in the host or its molting to the second instar. Parker and Wells (1919) however, reported that the first appearance of "grubs" under the skin was 9 to 12 days after oral infection. They go on to say that the skin was punctured two days later. At no point in their experimental descriptions do they state the stage of these "grubs". The valid interpretation of their experiments as to the duration of the first stage larvae cannot be obtained.

Under laboratory conditions, Parker and Wells (loc. cit.) found the pupal period to range between 47 and 173 days. However, Moilliot (loc. cit.) obtained a pupal period of 11 1/2 months in an outdoor insectary. Another example of a natural pupal period was from 8 to 10 1/2 months (Dalmat, 1943). The above workers claim that the large range of the pupal stage may be due to the existing moisture, temperature, amount of food stored in the puparia, and the time of year in which the pupation happened. Cases of a short pupal period may be during the mid-summer and a long example if started in the fall or winter.

B. ECOLOGY

As stated above the pupation of Cuterebra species may depend on environmental factors. These factors consequently could give rise to one or two generations a year, owing to the geographic location of cuterebrid flies. One approach to the problem of environmental factors has been the significance of soil in relation to pupation. Experiments carried out by Haugen (1942), Knipling and Brody (1940) employed sand as the soil surrounding the puparium. Methods employed by other workers (Parker et al., 1919 ; Cameron, 1926; Dalmat, 1942) did not classify the soil used in artificial pupation. The need for further investigation in this respect is warranted due to the paucity of specific data.

The factor of temperature in artificial pupation is another problem deserving attention. Knipling and Brody (loc. cit.) state "after the larvae had pupated, the jars in which they lay were placed in a cool closet." Many pupation experiments were carried out by workers without relating the conditions conducive to emergence. One substantial record of soil temperature, taken during pupation, was between 26 to 41° F (Dalmat, 1943). This temperature was reflective of a situation wherein the pupal jars were kept in an outdoor subterranean basement during the winter.

Moisture, according to Johnson (1930) is believed to be the prime factor necessary for pupation. He reported in

his study on the rearing of Cuterebra pupae that water was added intermittently to insure a moist condition. Others state that no water was added at any time prior to emergence (Greene, 1935; Hall, 1925). The different methods undertaken for pupation have created a need for further study on the factors affecting pupation.

Vegetation as an agent in the ecology of Cuterebra species has been mentioned in papers dealing with the life history of these flies. Townsend (1935) stated that eggs are laid on bark of trees and shrubs or stems of herbaceous plants which the hosts visit for feeding. Beamer (1950) observed an adult female laying eggs on the tips of celery grass near a rabbit runway.

Pertinent to the habitat of the adult flies, Cameron (1926) offered the information that the flies preferred dark situations, examples of which were outbuildings, summer kitchens and ground burrows of rodents. The females of Cuterebra, according to Townsend (loc. cit.) are frequently encountered flying close to the ground in open woodlands or perched on herbage in wooded lowlands.

Adult flies have been observed in flight from June to September (Cameron, loc. cit.). Cuterebrid larvae were found by Knipling and Brody (loc. cit.) in cottontails during June and November. However, records from the Kellogg Station show that hunters have reported the warbles in rabbits during other months of the year. Harkema (1936) discovered in his findings

of Cuterebra infestations in North Carolina cottontails that the percentage of hosts infested were highest in October, June and January respectively.

Curran (1934) indicated that these flies have been reared from rabbits, rats, mice and squirrels. Occasionally, they invade such animals as the cat and the dog, and even hogs, cattle, mules, opossum and man. Knipling and Bruce (1937) relate that larger animals, pigs, cows and mules, are not frequently found infected with cuterebrids and states that these infections are accidental.

Cuterebrid larvae have been found in various locations of their hosts. Examples of sites recorded were the neck, back, shoulders, belly, head and scrotum, especially in areas where the skin was thin. Cameron (1926) discovered that in the dog larval sites were particularly noted in the scapulo-humeral region.

An important problem arising in the study of the cuterebrid parasites is the effect of the larvae on their hosts. These effects range from no effect to cause of death. Because of certain species locating in the scrotum of the host, Haugen (1942) claimed that the cuterebrid larvae may cause sterility. Scott and Snead (1942) found a relationship between the peak in Cuterebra infections to a definite decrease in mouse populations. Gray squirrels in the North Carolina study of Allison (1953) were greatly affected in a like manner. Of economic importance is Lindquist's study (1937)

that Cuterebra species were the main predisposing agent of primary screworm infections in cottontail rabbits. Knipling and Bruce (loc. cit.) presented a similar situation in a mule. These same authors offered a case wherein the direct cause of death in a pig was due to a cuterebrid larva which was lodged in the trachea. Juvenile cottontails, have been found by Bruna (1952) to be greatly debilitated and weakened so that secondary effects such as predation or pneumonia may cause death. Cameron (loc. cit.) cited an example of anaphylactic shock in a dog undergoing extraction of larvae. During the extraction a larva was accidently crushed under the skin and shock ensued. Symptoms prevailed for seven hours and the animal recovered.

These robust bot flies are restricted to the western hemisphere. The Appendix offers the distribution ranges, the localities and bibliographic references of Cuterebra species concurrently with their hosts. These notes were summarized from papers of Townsend (1935), Knipling and Bruce (1937), Hall (1921), and Harkema (1936).

C. SYSTEMATICS

Townsend (1917) in his synoptic revision of the family Cuterebridae states "certain species of the older authors have been misidentified, certain valid species names have been put in synonymy, certain synonyms have been used as

valid names, and certain aberrant specimens of old species have been described as new species." This statement reflects the confusing early history of the systematics of Cuterebra species. The subsequent history is still uncertain due to the "lumpers" and "splinters" views on taxonomy. Also the above author presented a key which separated the genera of the family. Examples of genera were Pseudogametes, Dermatobia, Bogeria, Rogenhofera and Cuterebra.

The first reported note on Cuterebra was that of Fabricius (1776) in his reference of Oestrus buccatus. Oestrus was believed to be the original Cuterebra genus as later recorded by Brauer (1863). Fabricius' taxonomic description was maintained until 1815 when Clark designated it as Cuterebra purivora. Latreille (1816) changed the species to buccata which remained unchanged until Wiedemann (1830), through a misidentification, placed it in the genus Trypoderma. Macquart (1835) re-established it in its proper genus, that of Cuterebra. This final partition was confirmed by Joly (1846), and had held since. An error was noticed by Mik (1895) on the use of the name "Cutiterebra" in some reviews. Fortunately, this error was apprehended before confusion reigned again.

Another taxonomic example of unstableness was in the Cuterebra horripilum species. Clark (loc. cit.) in his study on bots maintained that Cuterebra cuniculi and C. horripilum were two distinct species. However, various other subsequent

taxonomists (Brauer, 1863; Townsend, 1917) offered evidences identifying the two species as horripilum. At present, the contention among contemporary taxonomists is that C. cuniculi is a synonym for C. horripilum. Townsend (loc. cit.) also presented the synonym of C. abdominalis for C. horripilum. Dalmat (1942) distinguished C. peromysci from C. fontinella Clark and the former species from C. fasciata Swenk. All above divisions have been accomplished by taxonomic studies on adult flies. For adult species identification, the reader is referred to reviews by Clark (1815), Brauer (1863), Swenk (1905), Jones (1906), and Townsend (1917). Supporting bibliographic references may be found in Appendix I. However, interests in this systematic study were in the investigation of larval forms.

Contemporary taxonomic studies of Cuterebra larvae are mainly those dealing with second and third stages. This is due to the insufficient sampling of first stage larvae necessary for valid characters (Knipling et al., 1940). Examples of first stage larval studies are those of Ferris (1920), Beachley and Bishopp (1942) and Dalmat (1942).

The general approach to a systematic study of cuterebrid larvae is the study of the following features: (1) the relative size and shape of spines, (2) the number and distribution of spines, (3) the structure and size of the posterior spiracles, and (4) general appearance of the larva. Knipling and

Brody (1940) stated that there were other characters of lesser importance, namely the size and shape of the larva, the general structure of the cephalopharyngeal mechanism, and the size and shape of the anterior spiracles.

The body of the first stage larva was shown to be fusiform, tapering at both ends and measurements on the early instars have been recorded by Ferris (loc. cit.) and Dalmat (loc. cit.) as 1.4 to 4.2 mm. in length and 3.2 mm. for the width. The latter author discovered that 12 segments were visible in this stage, and demonstrated cone-shaped dermal appendages. On the twelfth segment the posterior spiracular plate was found with each spiracle terminating in two slits. Records of the color of the stage were not given in the text of these authors.

However, in his study of the second instar, Dalmat (1942) stated that the body was white. The average length and width was 9.5 mm. and 3 mm. respectively. Cone-shaped and scale-like spines were seen covering the segments. Some cone-shaped spines pointed posteriorly and others pointed anteriorly. These spines were situated on the anterior and posterior aspects of a segment, and the direction of the spines was the reverse of their location. Cone-shaped spines, according to Cameron (1926), may be single or multipointed in certain larval species. Specific spinal characteristics for each of the twelve segments were presented by Dalmat (loc. cit.), along with measurements on the posterior spiracles.

The spiracles measured 0.12 mm. in length and 0.18 mm. in width. Knipling and Brody (1940) found in their investigations that the length and width of the spiracles was 0.30 mm. and 0.26 mm. It is probable that the latter authors had a later phase of that stage. After internal examination they found that the average length and width of the cephalopharyngeal skeleton was 1.6 and 0.75 mm. No measurements of spines were carried out in the studies cited above.

In studying the third instar, the degree of pigmentation of certain spines was recorded (Knipling et al., loc. cit.). They stated that pigmentation distinguished the relative age of the larvae, that is a newly molted larva from a mature larva. There was a lack of pigment in the former larva, and the more mature a bot became the darker the larva. Lengths of mature larvae ranged from 20 to 43 mm. and widths approximately equal to one-half the lengths. Spines were measured from base to tip to obtain lengths ranging from 0.36 to 0.80 mm. Base measurements for width ranged from 0.25 to 0.65 mm. These range variances were reflected in larvae of different Cuterebra species. A comparison of larval characteristics was presented by Knipling and Brody (1940) using Cuterebra buccata and C. cuniculi=(horripilum) as the bot examples.

The sampling material of cuterebrid larvae employed in the descriptions of the above workers was here discovered

to be rather limited. Thus need of a greater bot sample is necessary in order to achieve more finite characteristics.

The systematic study of pupae has been neglected by workers, probably because of the immediate fixing of mature larvae for future investigation. The puparium is essentially a dried hardened third stage larval skin, which has a more spherical shape, reflecting a stouter condition in the middle and posterior (Dalmat, 1942). Due to the shrinking effect, only ten segments are visible as the cephalic and spiracular segments are so retracted that they are not discernible. Anterior spiracles, everted during pupation, may be found between segments 2 and 3 of the puparium as two light brown, column-like tubercles projecting from the integument (Knipling and Brody, 1940). It was possible to retrogress by studying the morphology of a puparium with mature larval features, from which an identified adult fly emerged.

Dalmat (loc. cit.) stated that the emergence of the fly was brought about by the detaching of the dorsal aspect of the first 5 segments in a single piece often referred to as a cap. This method of ecdysis is typical of the dipteran suborder Cyclorrhapha.

Among the families of Diptera, and particularly in the Oestridae, a structure termed the "button" may be present in the mid-line of the posterior spiracular plate. This structure is essentially an accumulation of scars produced by the

discarded spiracles of the preceding stage. Greene (1925) stated that the Cuterebra larvae lack the button in the spiracular plates. However, papers by Knipling and Brody (1940) and Dalmat (1942) showed posterior spiracular plates with buttons present in the third instar. The resolution of this problem will be discussed later.

Of morphological significance is the length and width of the posterior spiracles and of the spiracular plate itself. In a third instar example cited by Dalmat (1942) the length and width of the posterior spiracle were 1.1 mm. and 0.5 mm. respectively. Two kidney-shaped spiracular plates, each containing three well defined sinuses, were 0.91 mm. long and 0.33 mm. wide.

It was hoped in this work that the various inquiries on the life cycle of cuterebrid species would be clarified. Instars of larvae were studied in the hosts to determine the seasonal periodicity, duration and incidences of the larvae. In these studies some effects of the larvae on the hosts were ascertained.

As a consequence of the life history study, ecological data was determined. Ecological factors such as soil and vegetation were examined in relation to the environment of Cuterebra. Modes of infection were studied from the above investigations.

Systematic investigations on cuterebrine larvae were carried out with the object of separating various Cuterebra

instars. Morphological similarities and differences of the larvae were discovered which were used in their species identification.

Confirmation of the larval species was accomplished in the taxonomic studies of pupal cases from which identified adult flies emerged.

MATERIALS AND METHODS

The choice of a cuterebrid infected host was founded on the facility of capture and handling as well as obtaining substantial numbers of hosts and parasites. Michigan cottontails, Sylvilagus floridanus mearnsii, reasonably fulfilled the study prerequisites.

Subsequent observations were made on individual hosts in order to follow the course of infection. In the continuous recapture of hosts, some questions on the life history of Cuterebra were clarified. However, these periodic studies were not so frequent as to interfere with the natural habits of the cottontails. The study area was carefully analyzed previous to the onset of trapping, and also while in the course of the project. Proposed areas for analyses were host burrows, nests, runways, and feeding areas.

Tabulation of data was done while in the field in order not to lose the freshness of ideas. Infected animals were brought into the laboratory under conditions similar to their habitat, and more complete studies were carried out. They were released into the study area as soon as possible to observe re-infection. Situations peculiar to the procurement of adult flies and larvae were established in the field. The approach to the problem of the life history and ecology

of Cuterebra was adapted as nearly as possible to natural conditions.

For the systematic study the collection of larval material gave an adequate sample of various instars. Inasmuch as larvae obtained from cottontails were allowed to complete pupation, additional larval specimens had to be obtained. Larvae were obtained from road kills, rabbits killed by hunters and trap-killed cottontails. Various other representative instars of larvae were collected from other hosts. Other sources of these examples were veterinarians and state conservation departments.

A. FIELD STUDIES

Of first concern was the mapping of the study area, in which vegetation, topography and soil was graphically recorded. The area under investigation was the state game farm near Mason, Michigan. The game farm functions principally as a pheasant farm, covering an area of 250 acres. However, the area offers excellent habitat for rabbits.

Trapping investigations began May 5, 1953. Thirty-two wire live traps were set in areas thought to be of rabbit use. Daily visits to the trap line were made from May 5 to November 24, 1953, and observations were recorded. A normal run of the trap lines would consist of an early morning inspection of the traps for captures, closely scrutinizing the vegetation

bordering rabbit runways for cuterebrid eggs, observations from a hidden area in the swales and wheat fields for adult flies and noticing rabbit movements closely. Wheat plants found on the edges of the fields were inspected for eggs. Beamer (1950) stated that the eggs were seen with the naked eye.

Six o'clock in the morning proved a fruitful hour to study rabbit movements. Field observations on the adult flies were made later in the day when the temperature was warmer.

Upon capture the rabbit was moved from the wire trap into a cloth bag and weighed. Rabbits were then immediately tagged in the ears. The tag number, age, sex of host, and area of capture were recorded. Cuterebrid larvae examinations were accomplished in the field while the animal was still in the bag. Areas of the neck, back, scrotum and shoulders were examined for warble formation, cuterebrid eggs and larval air holes. This examination consisted of a close scrutiny of the skin and a detailed palpation of the mentioned areas. Other areas of the body were generally observed.

The location, stage and number of bots on infected cottontails were noted, along with measurements of air holes when found. If the "bot" stage in the rabbit happened to be a second or a third instar, the infected rabbit was brought to the university laboratory for further study. A cottontail

with a mature larva was placed in an outdoor wire pen and observed. Dropped mature larvae were collected and effects on the host were summarized. Infected rabbits were released into nature a few weeks later, being temporarily held in order to study whether latent original infections would reach their final location.

The other method employed in the collection of cottontails was that of collecting highway killed specimens. This was carried out in the spring of the years 1953 and 1954 by means of early morning observations of various highways. Counties of Michigan studied were Ingham, Kalamazoo and Barry. Samples of cottontails at this time of the year were sufficient as rabbit movements were intensive during mating season. Autopsy protocols were carried out on these cottontails, in which the animals were first externally observed for warbles. Then the specimen was skinned in order to check subcutaneous larvae or remnants of larval scars. Nasal and oral cavities were examined as possible larval migratory routes. Internal examinations were made of the body cavities, mesenteries of the abdomen, the digestive tract and the musculature of the back, neck, fore and hind limbs. Scrota of males were dissected for larval stages. Specimens submitted by hunters in the fall at the Kellogg Forest, near Battle Creek, Michigan, were handled in the same manner. However, in the majority of the hunting cases, the larvae were extracted from

the hosts. These larval examples, depending on the stage of the parasite, were either kept for pupation or for mode of infection studies.

Mature larvae collected for pupation outdoors were placed in soils of various ecological situations. Fly traps were planted over the pupation sites. Thirteen sites were established by November 24, 1953 at which time rabbit trapping was terminated. These sites were routinely examined throughout the winter and spring to prevent damage or disturbance.

Fly traps were also employed during the summer of 1953 for the procurement of adult cuterebrid flies. Various baits were placed under the traps in the hope of luring and eventually capturing adult flies. Baits used were gasoline, vinegar and commercial rabbit lure. The above bait solutions were applied to the sponge of a "sweat band", and the band was wrapped around the trap, placing the soaked sponge under the funnel of the trap. Every three days the sponges were resoaked with the solutions. Fly traps were set in areas where adult flies might habit.

Mode of infection studies were carried out in the field during the summer of 1954. Inasmuch as areas of high Cuterebra infection were discovered from the previous year's study, bait pens, containing live cottontails were established there. Cottontails used in these studies were isolated for one month prior to placement in the bait pens in order to

insure a negative pre-experimental condition. Different ecological effects, such as experimental exposure to vegetation, lack of vegetation, field borders, swale edges, brush piles, outbuildings were individually attempted. Domestic white rabbits were also employed as controls in these outdoor situations. Bait animals exposed to vegetation were allowed to eat the prevailing vegetation. However, cottontails not having vegetation were fed commercial rabbit feed only. All animals were given water in an inverted animal cage drinking tube, for the purpose of avoiding possible cuterebrid egg contamination. Routine procedure of bait pens consisted of semiweekly examinations of the host and of the pen for phases of the life cycle of Cuterebra.

Concurrent with the Mason game farm study, a similar trapping program was started on August 10, 1953, under the supervision of Mr. Aelred Geis, at the Kellogg Station, near Battle Creek, Michigan. Though the program was later than the Mason area, it was partially compensated by the use of seventy-five live traps on 450 acres.

A duplicate cottontail study was again started at Mason on May 20, 1954, employing fifty live traps. This last trapping project terminated on November 20, 1954.

B. LABORATORY STUDIES

Cottontails with second or third stage larvae were brought into the laboratory, and were placed in animal cages. The fecal trays were filled with sand to a depth of 4 inches, in order to allow pupation of dropped mature larvae. Periodic investigations, every two days, consisted of recording the weight, the air hole diameter of the larva, the appearance or disappearance of larvae, and the time of healing of larval cysts after the mature larva dropped. Cottontails were held for one month in the laboratory to check for possible latent original infections. The hosts were then released into the study area and closely studied in subsequent captures for reinfections.

Some collected mature larvae were allowed to pupate in jars containing depths of sand. The depth varied from 1 1/2 to 5 inches. Observations on pupation were recorded. At the end of fall trapping 1954, thirty-nine pupation jars with gauze or muslin tops had been set up. Sixteen jars were placed in a cool damp basement at the game farm on September 10, 1954. Temperature was recorded throughout the winter until January 16, when the pupae were brought to the laboratory and left at room temperature. The remaining pupation jars were individually brought into the laboratory as soon as the pupae were established.

C. SYSTEMATIC STUDIES

The primary objective in this study was to collect mature larvae, allow them to pupate and to identify the adult flies obtained. Once the adults were known, the puparia were studied for larval taxonomic characteristics. In this manner known types could be established for future reference. However, inasmuch as the possibility of procuring successful pupations was rather small, larvae from various hosts were collected. In general, these latter larvae were received from outside sources in a preserved condition.

Hosts recorded from samples in fixative were dog, cat, white-footed mouse and jack-rabbit. Also they were from cottontails, peculiar to the states of Michigan, New Jersey, Pennsylvania and Wisconsin. These bots were segregated according to host and studied separately. Features studied were similar to those suggested by authors in the introduction.

Measurement of the larger spines, posterior spiracles and oral skeletons were taken with a compound microscope at 100 magnifications, using an ocular micrometer.

The method of preparing the stigmata for identification was carried out in the following manner. Since the larvae were hardened in alcohol for a number of days prior to receipt, the stigmata were rather firm and could be cut out. This dissection was best accomplished under the stereoscope with the aid of two microneedles and a pair of microscissors.

Anchoring the bot specimen firmly in a paraffin mold, the stigma was teased with the microneedles and excised intact with the microscissors. Stigmata from the first and second instars could then be passed through two 5-minute changes of 70 percent and absolute alcohol. The section was rinsed well in diaphane and a coverslip placed on a glass slide using diaphane as the mounting medium. Due to their dense pigmentation the stigmata of third stage larvae and puparia had to be cleared first in a boiling 10 percent KOH solution. Boiling was usually continued for an hour or two in order to obtain clear spiracular plates. After removal from the KOH solution, the stigmata were washed in water thoroughly, dehydrated in the usual manner and mounted in diaphane.

A similar protocol was used for the preparation of cephalopharyngeal skeletons. However, in order to obtain excellent view of the specimen, the skeleton was cut with microscissors at the hypostomal bridge and each section was mounted showing an outer lateral and inner lateral surface.

Areas of segments showing the shape and multipoints of spines were excised and mounted in diaphane.

The segments of the bots were closely studied for differentiating characteristics to separate the larvae as to species.

RESULTS

A. LIFE HISTORY

1. Obvious Appearance of Larvae in Their Hosts

It seemed that with the continuous recapturing of cottontails the development of larval stages in the hosts could be studied. Data gathered on the trapping of Mason rabbits in 1953 revealed the percent of recaptured rabbits to be 35 by November 7. With this recapturing certain facts on the timely appearance of larvae were noted. On August 1, 1953, a recaptured adult cottontail which was previously diagnosed externally as negative for Cuterebra July 10, 1953, was found to have an early third instar in the neck. Thus, it was only 22 days from a probable negative condition to an apparent third stage. This larval time period occurred in three infected recaptures. Another cottontail previously captured on July 21, 1953, and externally determined as negative for Cuterebra larvae, was recaptured on August 5 with an early third instar larva. Time for this larval example was 15 days. Various other examples of larval growths were: from negative to an early third instar, 26 days; from negative to a well healed larval wound (dropped mature larva), 39 days. The presented data reflected an obvious internal larval appearance in cottontails approximating the time of 22 days.

2. Anatomical Locations of Larvae Infections

Of forty-nine rabbits infected with Cuterebra larvae during the two year study at Mason, only one example diverted from the neck region. This single example was in the left front leg over the suprascapular region of the shoulder bone. In the Kellogg Station data of 1953 two cottontails were found showing unusual infection sites. One cottontail with four cuterebrid larvae had two of the larvae in the cheek region and two in the neck. The other had one larva on its back and another larva in the scrotum. The primary site of Cuterebra infections in 187 southern Michigan infected cottontails captured at the Kellogg Station and Mason Farm was in the neck.

3. Field Pupation Experiments

Eleven outdoor pupation sites were established in 1953 to determine times of emergences of adult cuterebrids. These sites were investigated throughout the winter and spring in order to maintain the fly-traps. In the late spring of 1954 the locations were frequently checked for newly emerged adult flies. Only one fly-trap had a positive result. The larva used in this trap was taken from a white-footed field mouse, Peromyscus leucopus, on August 27, 1953. On the very day of capture the mature larva pupated in the sandy soil

of the pupation site. A densely vegetated sumac border was the site of this fly trap. Date of emergence was July 25, 1954, which offered a pupation time of nearly 11 months. Dr. Sabrosky later identified the fly as a female Cuterebra fontinella. Longevity of this fly was only one day in a laboratory container. No studies on the modes of infection could be carried out with this cuterebrid fly. The remaining ten unsuccessful pupation sites were retained in the field until the spring of 1954. Though the pupae were discovered to be intact in the fall of 1953, no flies emerged the spring of 1954. Thus, the Cuterebra appeared in this study not to show a two-year cycle. Also it was found that one generation a year existed in Michigan.

4. Field Notes on Pupation

Mature third stage larvae were studied outside their hosts for times of survival. This extraneous situation was thrice recorded as 9, 10, 13 days before any serious changes began. This range of 9 to 13 days may be beneficial to the dropped mature larva in awaiting a suitable pupation.

Upon dropping on similar soil, mature third instars were noted to react in two ways. One manner was the immediate burrowing of the larva in the soil. On the other hand, other mature larvae merely laid on the soil surface with little locomotive action.

Burrowing time was noted to be in relationship with the type of soil. In sandy soil, five mature larvae revealed an average of 10 minutes. However, in sandy loam the average time for six mature larvae was 15 minutes. Edaphic areas recorded were solely those of light types such as sand and loam. Depths recorded on 27 burrowing larvae revealed ranged from 1 1/2 to 2 1/2 inches. There was no evidence of subterranean lateral migration by larvae during burrowing in three dozen soil jar containers used for pupation experiments.

5. Autopsies

Sixty-two cottontails were examined during the two-year rabbit study. These rabbits were autopsied throughout the possible Cuterebra cycle, that is from May to December. For external investigations examination areas for cuterebrid eggs were the lips, nose, neck, shoulders, abdomen, scrota, and heels of the hosts. Also in these areas the skin was closely examined for perforations which might be larval entry sites. Larval migratory routes, larval cysts or representative larval stages were sought in the internal investigations. The only positive prosecting was a discovery of a first instar cuterebrid larvae on June 28, 1953, which is discussed under first stage larvae seasonal periodicity. There were no other external or internal evidences of any phase of the Cuterebra life history in the autopsied animals.

6. Duration of Instars

Captured rabbits, having Cuterebra in situ were taken to the laboratory for further observation. Twelve examples were observed intermittently from July 25 to September 25, 1953. An animal which reflected the general time of cuterebrid larval stages was an adult female cottontail. Primary capture was on July 10, 1953, at which time the rabbit was not obviously infected. However, on August 1, 1953, it was infected by a mid third instar and in just eight days the mature larva dropped and a pupa was formed. Another example, an adult female rabbit, whose primary capture examination was negative on June 30, 1953, had an early third stage infection July 25. A mid third stage infection was recorded on July 31. The mature larva had dropped out and formed a pupa on August 8. Summarizing the laboratory larval data for 1953 and 1954 it was discovered that about 6 days elapsed from an early third stage to a mid third stage. Nine days was noted for the duration of a mid third instar to a pupa.

7. Air Hole Diameters

It was noted that upon checking cuterebrid larvae in the host, the air hole diameters gave significant data as to the stage of the larva. For example, air hole diameters for the early third stage larvae ranged from 3 mm. to 9 mm. No

diameters below 3 mm. were found in this study. Confirmation of this stage at the minimum point was made by excising the larva and identifying it. The larvae which were light brown in color had incomplete spiracular plates and intermediate spines which were low and not completely developed (see descriptions under Systematics). In the mid-third stage larvae the air hole range was from 10 mm. to 13 mm. At the 13 mm. diameter the larvae were observed in the laboratory specimens to be one-quarter out of their "larval cyst or bag". Diameters at the time of mature larval dropping were between 14 mm. and 16 mm. Usually the 17 mm. diameter showed a vacancy of the larval bag and a healing process beginning. No first or second stage larval air hole diameters were observed throughout the study. These larval air hole diameter averages were taken from sixty infected cottontails of which three cases are presented in Tables I, II, and III.

8. Mode of Infection Studies

With the trapping project of 1954 came the knowledge of what areas had the highest infestation of cuterebrid larvae. In these determined areas 22 live bait pens were established to discover the mode of infection. Two positive cuterebrid infestations were obtained. One location was a combination lumber and fence wire salvage dump. This salvage dump was bordered on the sides by a cornfield, evergreen

TABLE I

A LABORATORY AIR HOLE DIAMETER STUDY ON PHASES OF THIRD INSTAR CUTEREBRA, 1953

Host #144: Adult Male Cottontail																	
Date	6-28-53	7-24-53			7-31-53			8-9-53			8-12-53			8-14-53		8-16-53	
Larval #	Neg.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	2	
Larval Stage	Neg.	early 3rd	early 3rd	early 3rd	early 3rd	early 3rd	early 3rd	mid 3rd	mid 3rd	late 3rd	late 3rd	mid 3rd	dropped	pupa	late 3rd	dropped	
Air Hole Dia.(mm.)	Neg.	4	4	3.5	8	7	10	12	10	15	15	12	17	scar	15	17	
Days	0	26			7			9			3			2		2	
Rabbit Wt.(oz.)	47	50			45			44			44			45		44	

TABLE II

A LABORATORY AIR HOLE DIAMETER STUDY ON PHASES OF THIRD INSTAR CUTEREBRA, 1954

Host #405-406: Adult Male Cottontail																
Date	7-23-54			8-8-54			8-11-54			8-18-54		8-24-54		8-27-54		8-31-54
Larval #	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	3
Larval Stage	early 3rd	neg.	neg.	late 3rd	early 3rd	early 3rd	pupa	early 3rd	early 3rd	mid 3rd	early 3rd	late 3rd	mid 3rd	pupa	late 3rd	dropped
Air Hole Dia.(mm.)	3	0	0	17	3	4	scar	7	5	10	5	15	12	scar	14	15
Days		0		15	0	0		3			7		6		3	4
Rabbit Wt.(oz.)		34			33			33			33		33		38	41

TABLE III

A LABORATORY AIR HOLE DIAMETER STUDY ON PHASES OF THIRD INSTAR CUTEREBRA, 1954

Host #403-404: Juvenile Male Cottontail							
Date	8-5-54	8-8-54	8-11-54	8-14-54	8-18-54	8-19-54	8-24-54
Larval #	1	1	1	1	1	1	1
Larval Stage	early 3rd	early 3rd	early 3rd	mid 3rd	late 3rd	pupa	neg.
Air Hole Dia.(mm.)	3	5	6	12	16	17	scar
Rabbit Wt.(oz.)	23	23	24	24	24	26	29
Days	0	3	3	3	4	1	5

hillside and willow swale which had greenbriar and sumac intermingled on its border. It was in this swale that an adult cuterebrid was observed in the summer of 1953. The wire cage, measuring 8' x 3' x 3', was placed in the middle of the dump and was supported three feet off the ground. On June 10, 1954, a wild cottontail previously determined as negative by palpation and isolation was placed in the pen and was observed semiweekly for cuterebrid larvae. At no time was the animal in contact with natural vegetation. Also periodically examined for cuterebrid eggs were the nesting box and the rabbit cage. An early instar was discovered in the left side of the neck on July 11, 1954, with an air hole diameter of 3 mm. No eggs were found on the host nor in the pen. A mid third instar was reached July 28, 1954. The larva dropped from the host July 30, 1954, and was placed in a pupation jar where it pupated the next day. An autopsy carried out on the host revealed no evidence of migratory routes or other larval stages.

Location of the other positive bait experiment was in an area having a high larval incidence in 1953. This permanent Game Farm pen measured 50' x 15' x 8' and was exposed to natural vegetation. A rabbit burrow was supplied in the pen. Simultaneously, two adjacent pens containing white domestic rabbits were similarly established as controls. On June 17, 1954, two male juvenile cottontails originally determined as negative, again by palpation and 4 weeks

isolation, were placed in the cage. These were checked as in the previous bait experiments.

An early third instar was discovered in one juvenile cottontail on August 19, 1954. Previous to this date no positive signs of larval infection were noticed. Larval development was followed until maturity which was on August 27, 1954. The larva dropped from the host three days later and pupated on September 1. After the infection the host was sacrificed and autopsied, revealing no larval stages or migratory routes. No Cuterebra larvae were discovered in the domestic rabbits which were held in the cages until October 5, 1954.

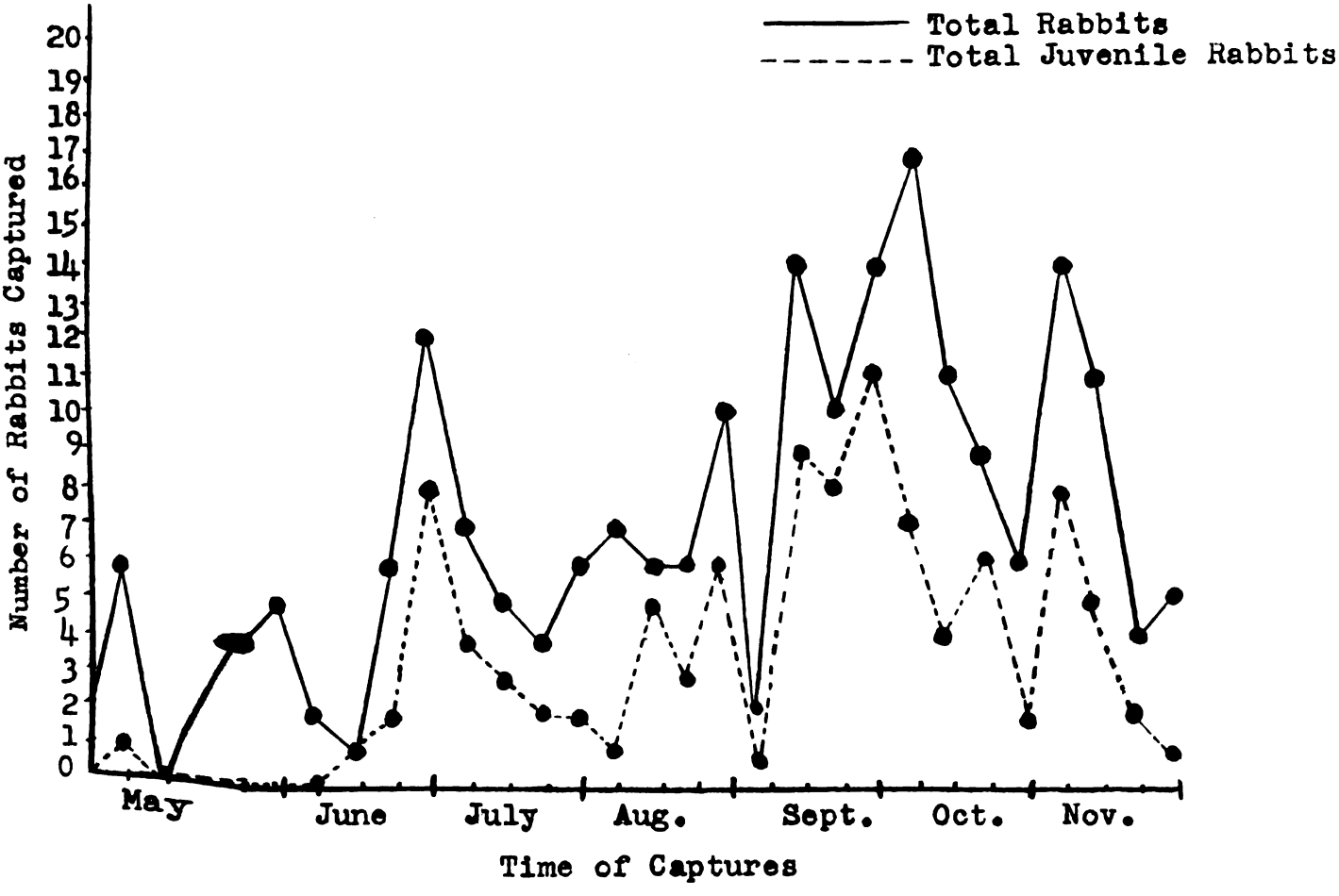
B. ECOLOGY

It was discovered that spring and summer trappings of Mason cottontails were rather difficult due, in part, to the availability and abundance of existing natural vegetation, (Graph I). This vegetation was more appealing as food than the field corn used as bait in the traps. Thus, the sampling data of wild cottontails was not great compared to numbers obtained in fall and winter. Later in the year there was an increase in captured rabbits due to the juveniles and a lack of natural vegetation which favors trapping.

Graph 1

Mason Trapping Record

1953



1. Incidence of Infected Rabbits on Mason Game Farm

The first trapping project began May 5 and ended November 24, 1953, at which time 138 captured cottontails resulted in 212 captures and recaptures. Because the curve of larval incidence occurred between July 19 and October 17 (Graph 2), only those rabbits captured during that time were considered. Within this time range 113 rabbits were trapped of which 29 had a cuterebrid infection (Table IV). Therefore the percentage of bot infestation was 26. In general, the infections were slightly greater in adults than in juveniles, however the periodicity of infections varied according to age groups. Juveniles had an incidence peak from August 2 to 15 whereas the adult pattern reflected "up and down" cycles throughout the project. The summer of 1953 was markedly and constantly high in temperature offering what was believed to be favorable conditions for cuterebrid infestations.

Incidence of Cuterebra larvae in the Mason game farm cottontails captured in 1954 was 29 percent. Number of rabbits handled during the infective period were only 57 (Table V). In part because of biological knowledge of the study area obtained from the previous year's investigation, there was a high spring collection of rabbits (Graph 3). However, as the trapping continued low numbers of cottontails were obtained due to the high juvenile mortality observed in the field. Inclement summer weather was believed contributory

Graph 2

Incidence of Cuterebra larvae

in Mason Game Farm Cottontails 1953

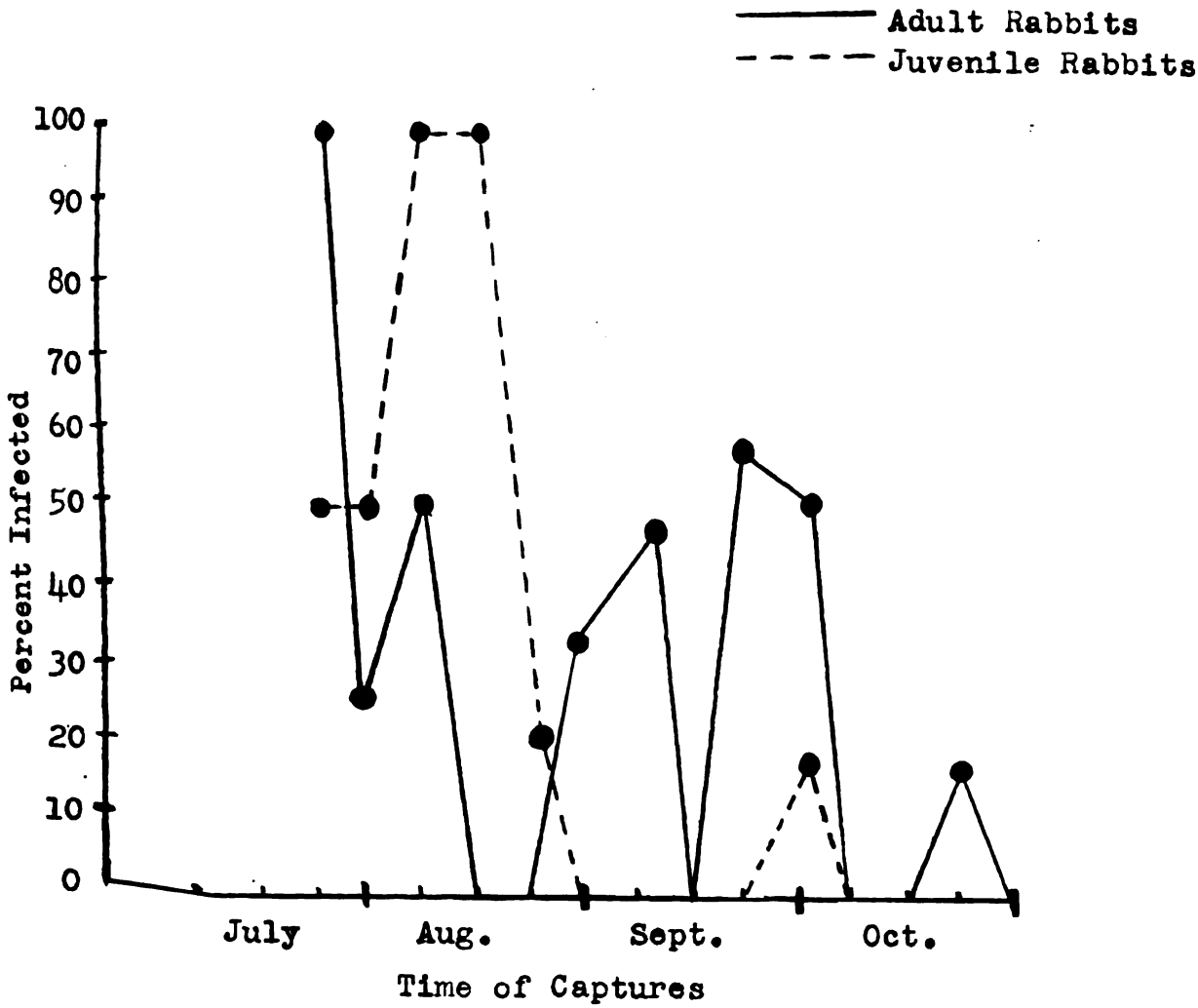


TABLE IV

INCIDENCE OF CUTEREBRA LARVAE IN MASON GAME FARM COTTONTAILS, 1953

	Time of Captures													
	July 19-25	Jul. 26 -Aug. 1	Aug. 2-8	Aug. 9-15	Aug. 16-22	Aug. 23-29	Aug. 30 -Sept. 5	Sept. 6-12	Sept. 13-19	Sept. 20-26	Sept. 27 -Oct. 3	Oct. 4-10	Oct. 11-17	Total
# handled	4	6	6	7	6	6	10	2	14	10	14	17	11	113
# adult rabbits	2	4	4	6	1	3	4	1	5	2	3	10	7	52
# juvenile rabbits	2	2	2	1	5	3	6	1	9	8	11	7	4	61
# adults with larvae	2	1	2	0	0	1	1	0	0	0	0	0	1	8
# adults with holes only	0	0	0	0	0	0	1	0	3	1	0	0	0	5
# adults not infected	0	3	2	6	1	2	2	1	2	1	3	10	0	33
# juveniles with larvae	1	1	2	0	1	0	0	0	0	1	0	0	6	12
# juveniles with holes only	0	0	0	1	0	0	0	0	0	1	0	0	0	2
# juveniles not infected	1	1	0	0	1	3	6	1	9	7	11	7	4	51
% total with larvae	75	33	66	14	17	17	10	0	0	10	0	0	9	17
% total with larvae or holes	75	33	66	14	17	17	20	0	21	10	0	0	0	26
% adult with larvae	100	25	50	0	0	33	25	0	0	50	0	0	14	15
% adult with larvae or holes	100	25	50	0	0	33	50	0	60	50	0	0	14	25
% juveniles with larvae	50	50	100	0	20	0	0	0	0	13	0	0	0	20
% juveniles with larvae or holes	50	50	100	100	20	0	0	0	0	13	0	0	0	22

TABLE V

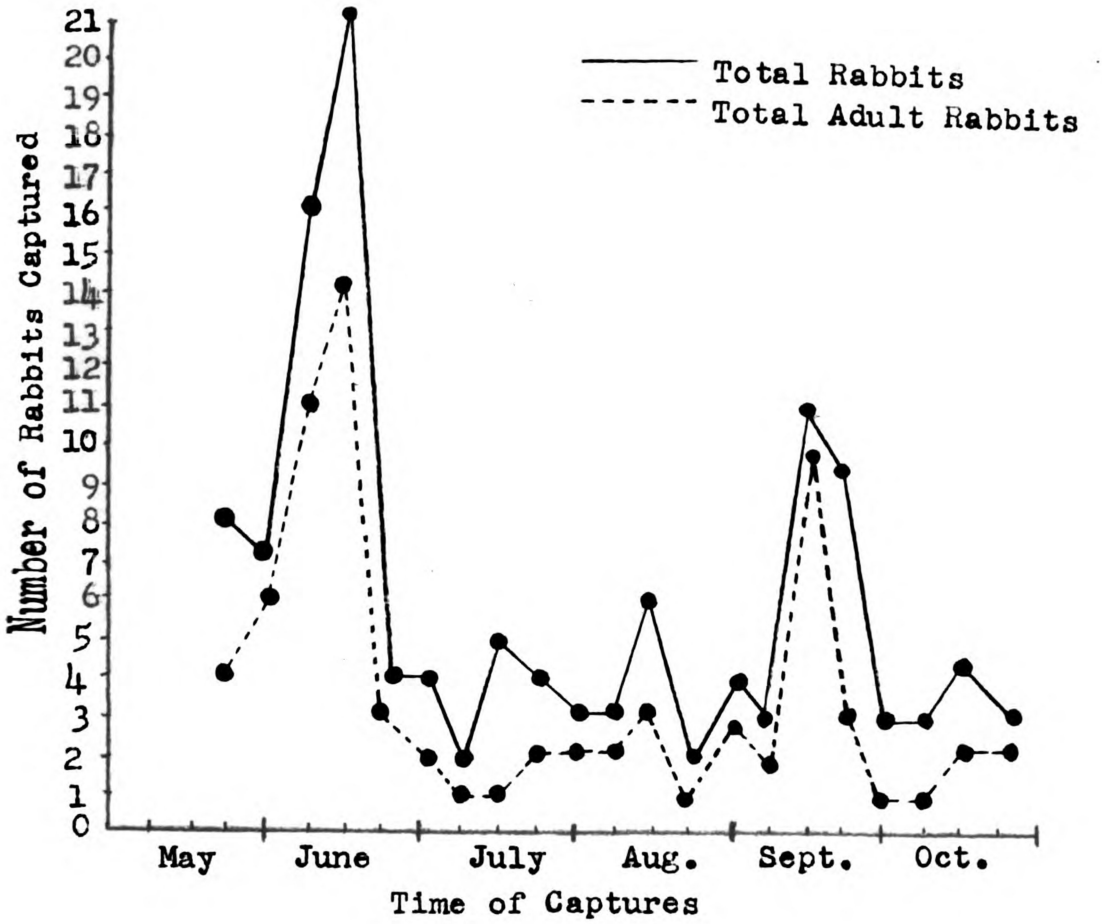
INCIDENCE OF CUTEREBRA LARVAE IN MASON GAME FARM COTTONTAILS, 1954

	Time of Captures													
	July 19-25	Jul. 26 -Aug. 1	Aug. 2-8	Aug. 9-15	Aug. 16-22	Aug. 23-29	Aug. 30 -Sept. 5	Sept. 6-12	Sept. 13-19	Sept. 20-26	Sept. 27 -Oct. 3	Oct. 4-10	Oct. 11-17	Total
# handled	3	3	3	6	3	3	3	11	9	3	3	4	3	57
# adult rabbits	2	1	2	3	2	2	2	10	3	1	1	2	2	33
# juvenile rabbits	1	2	1	3	1	1	1	1	6	2	2	2	1	24
# adults with larvae	1	1	1	0	1	1	0	0	0	0	0	0	0	5
# adults with holes only	0	0	0	3	0	1	0	3	1	0	0	0	0	8
# adults not infected	1	0	1	0	0	1	2	8	2	1	1	2	2	21
# juveniles with larvae	0	0	1	0	0	1	0	0	2	0	0	0	0	4
# juveniles with holes only	0	0	0	0	0	0	0	0	0	0	0	0	0	0
# juveniles not infected	1	2	0	0	0	0	1	1	4	2	2	2	1	16
% total with larvae	33	100	66	0	33	66	0	0	22	0	0	0	0	16
% total with larvae or holes	33	100	66	50	33	66	0	22	33	0	0	0	0	29
% adult with larvae	50	100	50	0	50	50	0	0	0	0	0	0	0	15
% adult with larvae or holes	50	100	50	100	50	50	0	22	11	0	0	0	0	39
% juveniles with larvae	0	0	100	0	0	100	0	0	22	0	0	0	0	16
% juveniles with larvae or holes	0	0	100	0	0	100	0	0	22	0	0	0	0	16

Graph 3

Mason Trapping Record

1954



to this situation. Additional factors were changing ecological and agricultural conditions which were believed due, in part, to the farm crops program established at the Mason Farm that summer.

The peak of incidence of juvenile rabbits in 1954 (Graph 4) was found to be on August 2 which would correlate with the peak obtained in 1953. Also comparatively similar were the incidence peaks relative to adult rabbits. In the later phases of the curves, minor peaks were noted indicating the appearance of emergent adult flies which continually arose from puparia during the summer and fall. These newly emerged Cuterebra adults would propagate new larval infections resulting in new minor cycles and peaks.

The number of warbles per infected rabbit was generally found to correspond with the peak of incidence (Tables VI and VII). This is explained by the concentration of larvae at that time which would lead to greater infections on individual rabbits.

2. Incidence of Infected Rabbits on the Kellogg Station

All larval material presented from the Station was carried out by A. Geis. Cuterebrid larvae infections in the Kellogg cottontail sample of 1951 were highest on juvenile rabbits during August 11 and 31 (Graph 5). Adult cottontails showed a peak in bots between September 1 and 10. Table VIII relates the percent of cuterebrid larval infections

Graph 4

Incidence of Cuterebra larvae

in Mason Game Farm Rabbits 1954

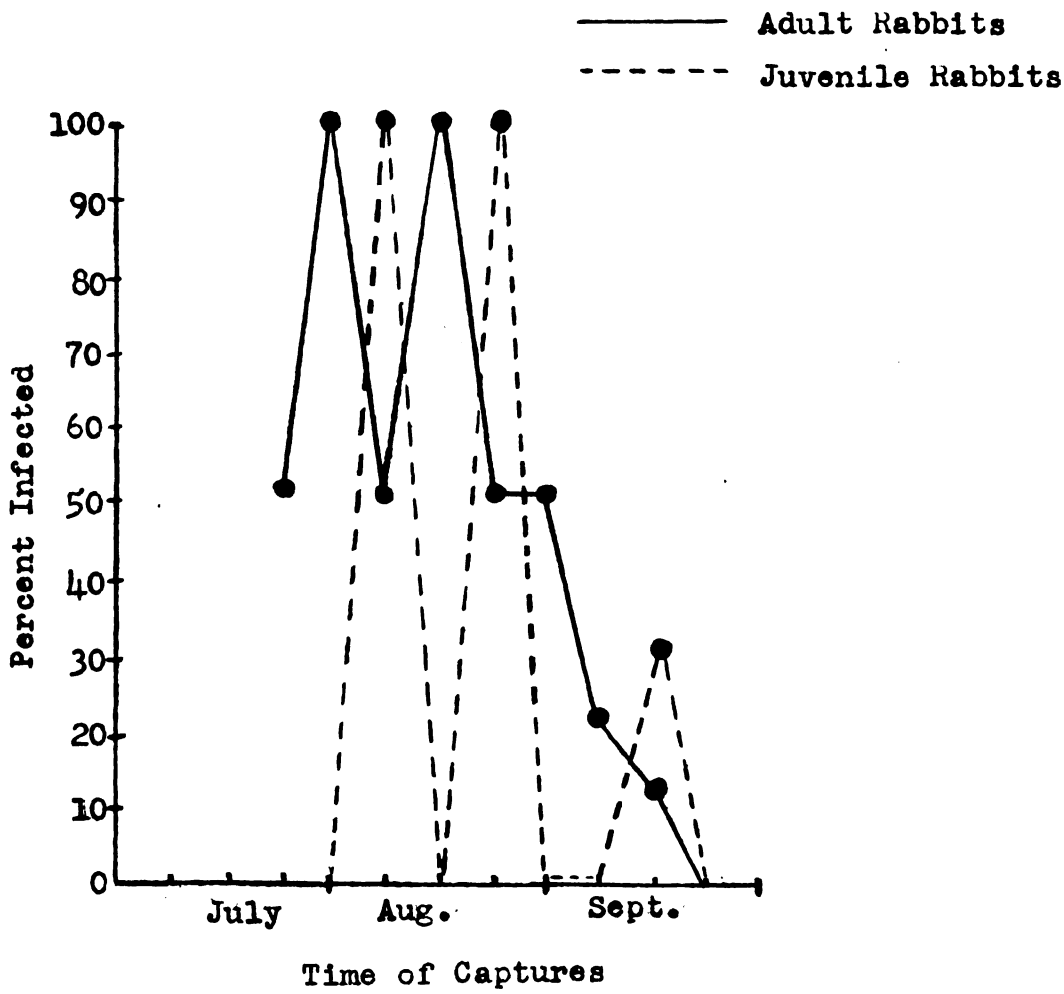


TABLE VI

NUMBER OF WARBLES PER INFECTED RABBIT
MASON GAME FARM, 1953

Date (1953)	Adults				Juveniles			
	Number of Warbles or Holes				Number of Warbles or Holes			
	1	2	3	Aver.	1	2	3	Aven
July 19-25	1		1	2	0			0
July 26-Aug. 1	1			1	1			1
Aug. 2-8	0	2		2	2			1
Aug. 9-15	0			0	1			1
Aug. 16-22	0			0	1			1
Aug. 23-29	1			1	0			0
Aug. 30-Sept. 5	2			1	0			0
Sept. 6-12	0			0	0			0
Sept. 13-19	3			1	0			0
Sept. 20-26	1			1	1			1
Sept. 27-Oct. 3	0			0	0			0
Oct. 4-10	0			0	0			0
Oct. 11-17	1			1	0			0
Total	10	2	1	1.2	6	0	0	1

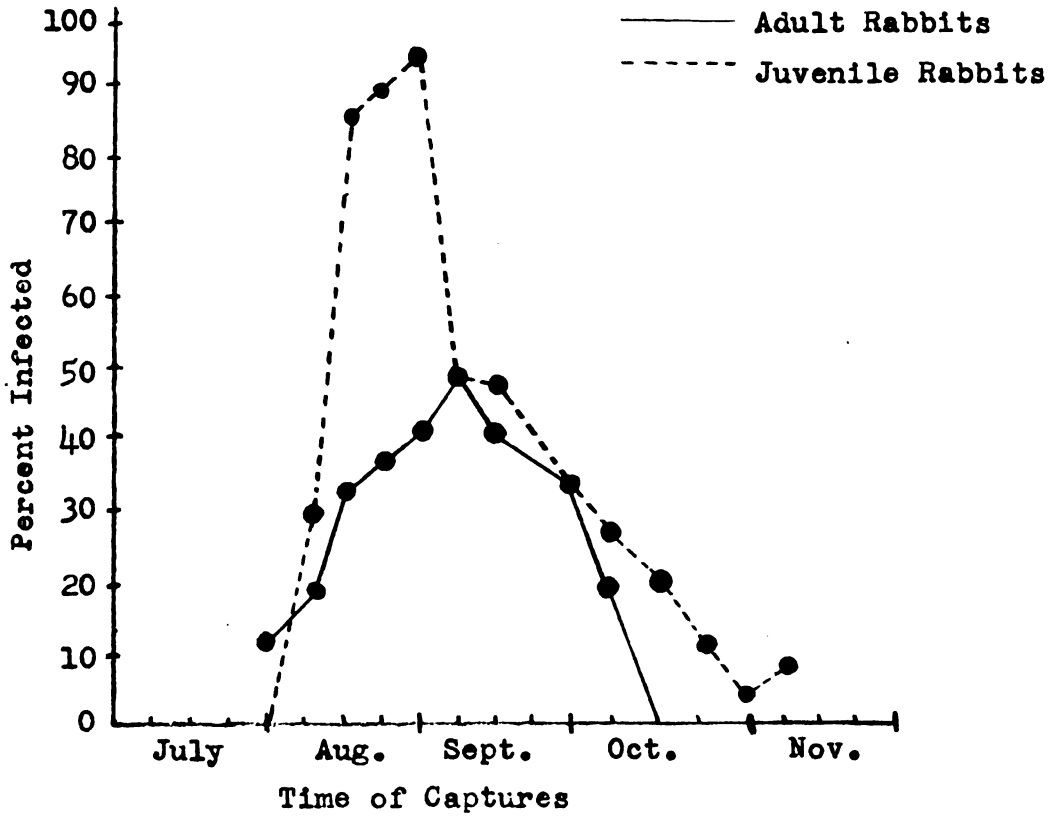
TABLE VII

NUMBER OF WARBLES PER INFECTED RABBIT
MASON GAME FARM, 1954

Date (1954)	Adults			Juveniles		
	Number of Warbles or Holes			Number of Warbles or Holes		
	1	2	Average	1	2	Average
July 19-25	1		1			
July 26-Aug. 1	1		1			
Aug. 2-8	1		1	1		1
Aug. 9-15	1		1			
Aug. 16-22		1	2			
Aug. 23-29		1	2		1	2
Aug. 30-Sept. 5	0		0			
Sept. 6-12	3		1			
Sept. 13-19	1		1	2		1
Sept. 20-26	0		0			
Sept. 27-Oct. 3	0		0			
Oct. 4-10	1		1			
Oct. 11-17	0		0			
Total	10	2	1.2	3	1	1.3

Graph 5

Incidence of Cuterebra larvae in
Kellogg Station Cottontails, 1951*



*From A. Geis

TABLE VIII

INCIDENCE OF CUTEREBRA LARVAE IN KELLOGG STATION COTTONTAILS, 1951*

	Time of Captures											Total
	July 20-30	Aug. 1-10	Aug. 11-20	Aug. 21-31	Sept. 1-10	Sept. 11-20	Sept. 21-30	Oct. 1-10	Oct. 11-20	Oct. 21-30	Nov. 1-10	
#handled	12	17	19	15	6	26	36	50	29	36	26	272
# adult rabbits	8	5	6	5	2	5	3	10	2	4	2	52
# juvenile rabbits	4	12	13	10	4	21	33	40	27	32	24	220
# adults with larvae	1	1	2	1	0	0	0	1	0	0	0	6
# adults with holes	0	0	0	1	1	2	1	1	0	0	0	6
# adults not infected	7	4	4	3	1	3	2	8	2	4	2	40
# juveniles with larvae	0	4	7	7	2	9	4	8	2	1	2	46
# juveniles with holes	0	0	4	2	0	1	7	3	4	0	0	21
# juveniles not infected	4	8	2	1	2	11	22	29	21	31	22	153
% total with larvae	8	29	47	53	33	35	9	18	7	3	8	19
% total with larvae or holes	8	30	63	73	50	46	33	26	21	3	8	29
% adult with larvae	12	20	33	20	0	0	0	10	0	0	0	11
% adult with larvae or holes	12	20	33	40	50	40	33	20	0	0	0	23
% juveniles with larvae	0	33	54	70	50	43	12	20	7	3	8	20
% juveniles with larvae or holes	0	33	85	90	50	48	33	28	22	3	8	30

*From A. Geis.

to be 29. This was determined from a total rabbit population of 272 which revealed larval infections in 79 cottontails. In this survey more juveniles had larvae than did adults.

In Table IX the larval incidences for 1952 give a total percentage of larval infections as 19 which is quite low compared to results in 1951. The rabbit sample for the later year was 191 of which 38 cottontails were infected. There was no evidence in Graph 6 of a definite peak of larval incidence for juvenile rabbits. However, it did appear that the juvenile curve was in the late phase of its infection, that is, it may show only the tail end of the curve. The adult cycle related a peak during September 1 and 5 which did compare chronologically with the data for 1951 on the same area.

A situation similar to the above seems to exist for the juvenile curve of the 1953 Cuterebra larval incidence (Graph 7). This curve exhibited a downward trend phase. Though the adult example did not show a peak as high as the past years, nevertheless there was a peak occurring between September 1 and 10 which did approximate the condition found in 1952. Twenty-one cottontails from a total population of 149 were infected with Cuterebra resulting in a 14 percent infection. This percent infection recorded in Table X was the lowest obtained in all the larval studies undertaken at the Kellogg Station. Essentially, the number of

TABLE IX

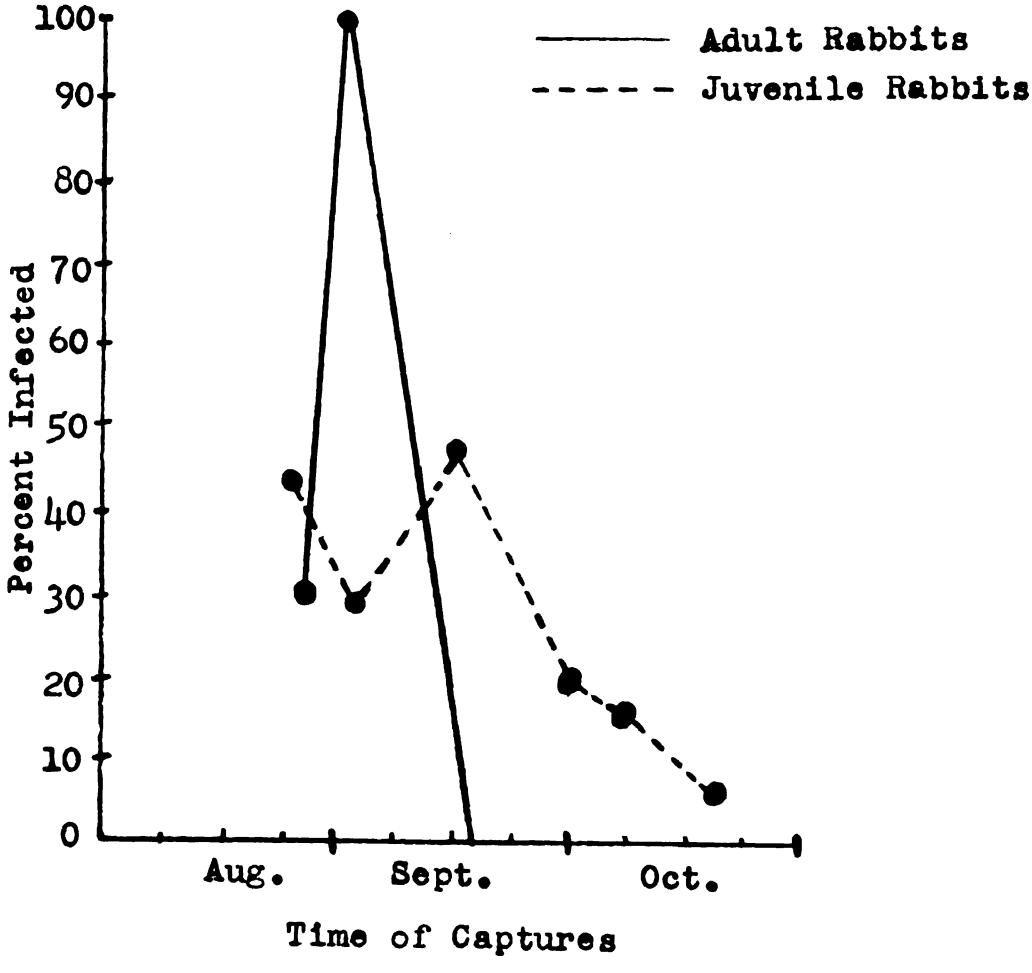
INCIDENCE OF CUTEREBRA LARVAE IN KELLOGG STATION COTTONTAILS, 1952*

	Time of Captures						Total
	Aug. 26-31	Sept. 1-5	Sept. 17-20	Sept. 21-30	Oct. 1-10	Oct. 11-20	
# handled	16	17	15	52	51	40	191
# adult rabbits	6	2	2	8	4	3	25
# juvenile rabbits	10	15	13	44	47	37	166
# adults with larvae	1	1	0	0	0	0	2
# adults with holes	1	1	0	0	0	0	2
# adults not infected	4	0	2	8	4	3	21
# juveniles with larvae	3	4	5	7	2	1	22
# juveniles with holes	1	0	1	5	4	1	12
# juveniles not infected	6	11	7	32	41	35	132
% total with larvae	25	29	33	13	4	2	12
% total with larvae or holes	38	35	40	23	12	5	19
% adult with larvae	17	50	0	0	0	0	8
% adult with larvae or holes	33	100	0	0	0	0	16
% juveniles with larvae	30	27	38	14	4	3	13
% juveniles with larvae or holes	40	27	46	16	13	5	12

*From A. Geis.

Graph 6

Incidence of Cuterebra larvae in
Kellogg Station Cottontails, 1952*

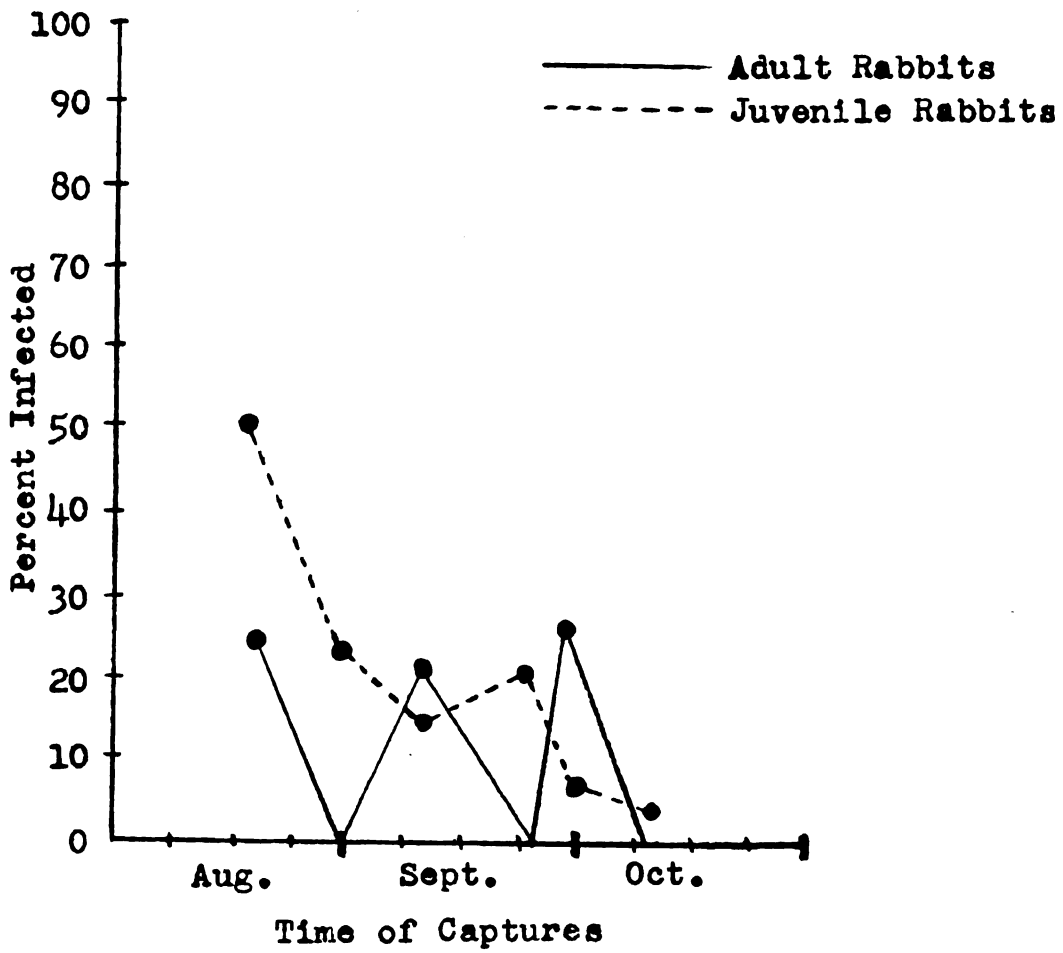


*From A. Geis

Graph 7

Incidence of Cuterebra larvae in

Kellogg Station Cottontails, 1953*



*From A. Geis

TABLE X

INCIDENCE OF CUTEREBRA LARVAE IN KELLOGG STATION COTTONTAILS, 1953*

	Time of Captures						Total
	Aug. 11-20	Aug. 21-30	Sept. 1-10	Sept. 11-20	Sept. 21-30	Oct. 1-10	
# handled	16	15	13	15	36	54	149
# adult rabbits	4	2	5	2	8	6	27
# juvenile rabbits	12	13	8	13	28	48	122
# adults with larvae	1	0	0	0	2	0	3
# adults with holes	0	0	1	0	0	0	1
# adults not infected	3	2	4	2	6	6	23
# juveniles with larvae	5	3	1	1	1	1	12
# juveniles with holes	1	0	0	2	1	1	5
# juveniles not infected	6	10	7	10	26	46	105
% total with larvae	38	20	8	7	8	2	10
% total with larvae or holes	44	20	15	20	11	4	14
% adults with larvae	25	0	0	0	25	0	11
% adults with larvae or holes	25	0	20	0	25	0	11
% juveniles with larvae	42	23	13	8	4	20	10
% juveniles with larvae or holes	50	23	13	20	7	4	14

*From A. Geis.

warbles per infected rabbit shown in Table XI was greatest when there was a peak in the incidence of Cuterebra larvae among the cottontails.

Graph 8 represents a composite picture of the averages obtained from the larval incidence studies carried out at the Kellogg Station and the Mason Game Farm. This was accomplished by chronologically tabulating the incidence percentages resulting from the individual studies and finding the percent averages therefrom. These results mainly strengthen the previously presented fact that the peak of larval incidence among southern Michigan juvenile cottontails is found between August 1 to 20. Also that the adult rabbit incidence curve presents an opposite condition wherein the major peak of incidence occurs between July 20 to 30, along with the secondary peak between September 1 to 10. Slight variances in peak incidences between the two study areas and various years are probably due to fluctuations brought about by sampling material and ecological factors.

The period before a peak incidence in larval infections as seen in Graphs 1 and 3 showed that few juvenile cottontails were captured. Field observations during this time revealed cottontails hiding during the hot July days in dark densely vegetated areas and in nests or burrows. After this "periodic hiding" the percentage of Cuterebra infected young cottontails captured is quite high. Perhaps

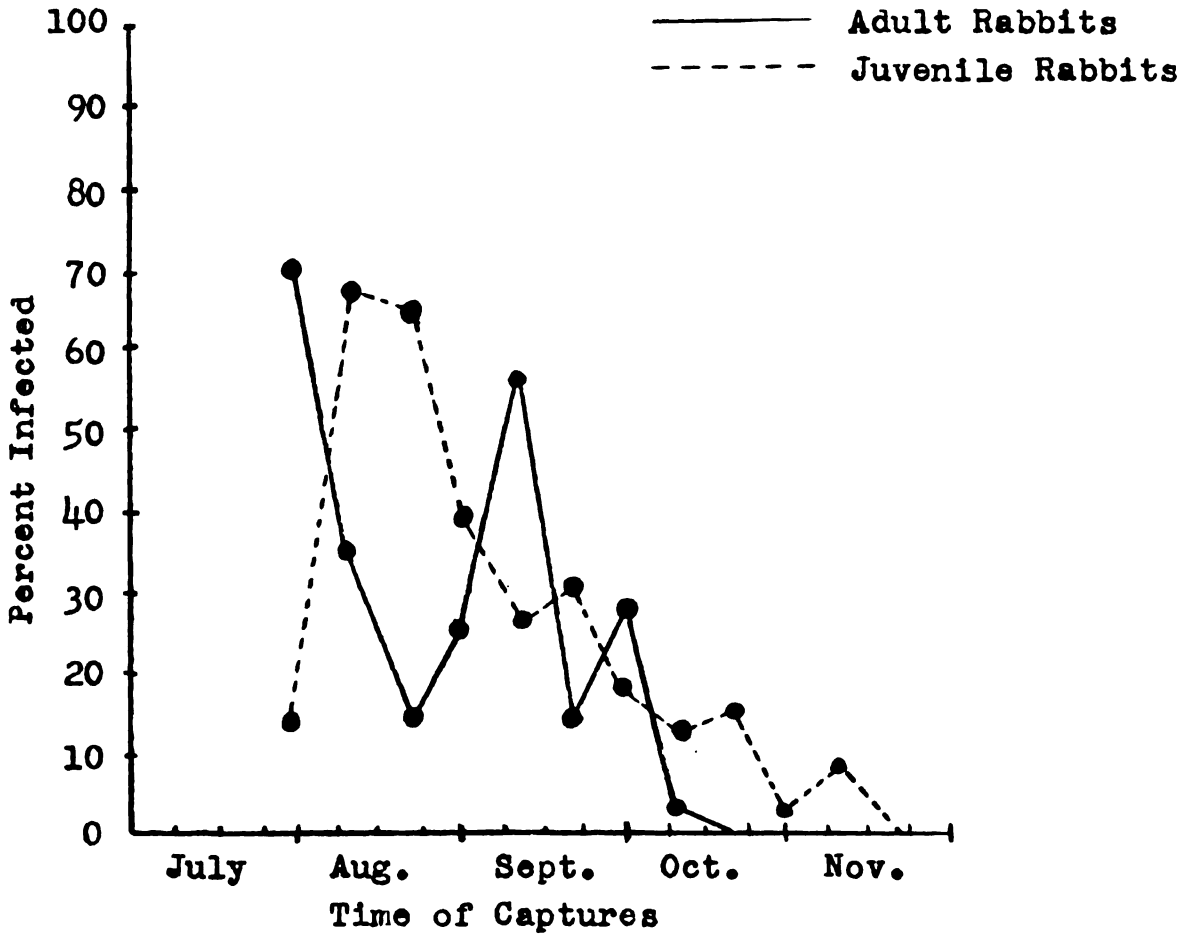
TABLE XI

NUMBER OF WARBLES PER INFECTED RABBIT
KELLOGG STATION, 1951, 1952

Date (1951)	Adults					Juveniles				
	Number of Warbles or Holes					Number of Warbles or Holes				
	1	2	3	4 or more	Aver.	1	2	3	4 or more	Aver.
July 20-30	1				1.0	0				0
Aug. 1-10	1				1.0	2		2		2.0
Aug. 11-20	1	1			1.5	4	4	3		1.9
Aug. 21-31	2				1.0	6		2	1 had 8	2.2
Sept. 1-10	1				1.0	1		3		2.5
Sept. 11-20	2				1.0	6	3		1 had 4	1.6
Sept. 21-30	1				1.0	7	3	1		1.5
Oct. 1-10	2				1.0	10	1			1.1
Oct. 11-20	0				0	6				1.0
Oct. 21-30	0				0	1				1.0
Nov. 1-10	0				0	2				1.0
Total	11	1			1.1	45	11	11	1-8 1-4	1.6
(1952)										
Aug. 26-31	1	1			1.5	3	1			1.2
Sept. 1-5	2				1.0	4				1.0
Sept. 17-20	0				0	5		1		1.3
Sept. 21-30	0				0	9	1	1	1 had 4	1.5
Oct. 1-10	0				0	5	2			1.3
Oct. 11-20	0				0	2				1.0
Total	3	1			1.2	28	4	2	1-4	1.3

Graph 8

Incidence of Cuterebra larvae in Southern Michigan Cottontails from 1951 to 1953



this hiding may be conducive to cuterebrid infections inasmuch as adult flies prefer dark situations and nesting areas (Cameron, 1926).

3. Incidence of Larvae in Sex of Rabbits

Results tabulated in this survey showed that the male cottontails have an incidence of cuterebrid larvae approximately twice as high as the female rabbits (Table XII). The month of August is the primary month of infection which would compare with the seasonal larval incidences. This higher occurrence in male rabbits may be partly explained by the activity of males in searching for mates during breeding seasons, thus offering a greater captured sample. Also in all probability more female cottontails, either pregnant or with young in nests, are less likely to wander under those conditions.

4. Seasonal Periodicty of First Stage Larvae

There is no mention in the literature relative to the periodicity of first instar larvae in Michigan cottontails. The first example of a cuterebrid infection in rabbits was a highway kill that was found in Ingham county near Mason, on June 28, 1953. Upon skinning, a first instar was discovered in the region of the neck immediately under the

TABLE XII

INCIDENCE OF CUTEREBRA LARVAE IN SEX OF MASON COTTONTAILS, 1953 and 1954

Date	1953		1954	
	Male	Female	Male	Female
July	5	3	3	2
August	7	3	6	2
September	6	3	5	2
October	1	1	0	0
Total	19	10	14	6

skin enclosed in a nodule approximately 11 mm. in diameter. Close observation of the infected area revealed no larval bag or air hole. Measurements on the larva were 5 mm. long and 2 mm. wide. Length of larval life was one day in a Petri dish.

Examples of other first instar larvae were from preserved material sent in to the laboratory. These were from veterinary sources and the hosts were domesticated cats. Dates of collection were recorded in the months of January, August and September.

5. Seasonal Periodicity of Second Stage Larvae

The first recorded second instar in Mason cottontails was on July 25, 1953. A swelling under the neck was palpated in an adult rabbit and the warble was extricated from the bag by a scalpel. The nodule measured 15 mm. in diameter and the larval measurements were 10 mm. long and 4 mm. wide. Upon microscopic examination it was determined to be a late second stage cuterebrid larva. No air holes were discernible. In Table II there is noted a cottontail which revealed subsequent larval infection not originally recorded. On July 23 the host was found to have an early third instar on the left side of its neck. The animal was brought into the laboratory and isolated in a clean cage. A large swelling, measuring 25 mm. in diameter was observed on

July 30 on the right side of the neck. No internal investigation of the swelling was carried out for fear of damaging the parasite. Larval development was allowed to continue through August 8 when two early third stage larvae with air holes appeared in the region of the tumor. It was believed that the stage of the larvae within the tumor was that of a second instar. The reasons for this conjecture were due to the measurements of the tumor and palpation of the larvae, supported by the fact that in the two-year screening study of approximately 450 cottontails for cuterebrid larvae, no air holes were ever found for second stage larvae.

Second instar periods recorded in preserved specimens, having Felis domestica as hosts, were in January, February, March and in June, besides the normal months described in cottontails. These stages were collected by veterinary students and were submitted with limited history on their hosts. Previous geographic locations of the hosts that may have determined unseasonal acquisitions of cuterebrid infections were not obtained.

6. Seasonal Periodicity of Third Stage Larvae

Recorded in Table I are examples of third instars in Mason rabbits which were discovered on July 24, 1953. Periodical development of these larval examples were followed in days relative to the growth of the phases (early, mid or late)

in the same table. The majority of third stages occurred during the middle of August and early September when the larvae were obvious as indicated in Graphs 2, 4, 5, 6 and 7. However, third instar examples in Kellogg Station cottontails were seen as late as November and December. Larval material from cats had representatives in nearly every month of the year.

7. Studies on the Adult Fly

The first adult cuterebrid was seen on July 4, 1953. At 10 A.M. the yellow-sided Cuterebra was observed flying low over a clover field. Unable to apprehend the fly, a thorough search of the clover plants under the flight area was carried out in the hope of finding eggs. Results were negative. A small white-sided Cuterebra was noticed in a thick willow swale on July 11 at 11 A.M. The fly appeared to be resting not quite a foot off the ground on young willows. Lazily it flew from willow to willow, intermittently resting on grass leaves. Upon approaching the fly, which was only a few feet away, with an open insect net, it swiftly darted into the dark core of the swale. The area revealed no eggs. On August 11, 1954, at 2 P.M. a yellow-sided cuterebrid was seen in a dense sumac area. The fly was captured and placed in a laboratory container for study. Three days was the length of life of this fly. It was later identified as an adult male

Cuterebra horripilum by Dr. C. W. Sabrosky. A significant fact afterwards discovered was that the three observation areas reflected a high incidence of Cuterebra infections.

8. Fly-baiting Experiment

Fly traps with bait were set near swales or other areas of suspected cuterebrid habitat in order to capture adult flies. Hindered by adverse weather conditions, baiting was practically ineffective. Baits would either be washed off by rain or evaporate after a few days exposure. Various baits used were: from July 4-6, rabbit lure; July 6-8, honey; July 8-10, rabbit feces in urine; July 10-12, gasoline; July 12-30, vinegar. Live bait, that is, cottontail rabbits in cages were employed from July 3-7; July 22-August 4; September 4-24 in areas believed having a high incidence of Cuterebra. The end result of all the above experiments carried out that summer of 1953 at the Mason farm was negative. No fly-baiting experiments were undertaken at the Kellogg Station.

However, on July 24, 1954, an adult cuterebrid fly was found in a fly-trap that had been placed over a rabbit burrow. This burrow was in a dark situation under a lumber pile in a salvage dump. The specimen was sent to Dr. Sabrosky who determined it as Cuterebra horripilum.

9. Laboratory Pupation Experiments

Thirty-nine pupation jars were established during the late summer and early fall of 1954. Various types of soil were used in order to study the ecological significance of soil on pupation. Soils varied from loamy clay to sand. By July 6, 1954, five adult flies were obtained through these experiments. In all five cases sandy soil was involved.

Emergence of these flies was noted to occur in a chronological manner, that is the first pupated example was also the first emerging fly. This first example pupated on August 1, 1954, and the date of emergence was March 3, 1955, resulting in a total pupation period of 174 days. Remaining pupae reflected total pupation periods of 175, 218, 234, and 235 days. The amount of water added to these pupation jars was 25 cc. per month. Four of the five positive pupation experiments were placed in a damp basement during the first four months of pupation. Ranges of temperatures during the winter months in the basement were 32 to 47° F. Past this time they were in the university laboratory at room temperature. The remaining pupa was continually in the laboratory, but situated on a window ledge partially exposed to winter conditions. Conditions existing at emergence were that it occurred during the day and in a very hot sunlight. Summarizing these pupation data, it appeared that a slight exposure to cold stimulated the pupae towards an early

pupation period. The depth of soil in the pupation jars did not generally bother pupation as results were obtained in sandy soil in as little as 1 to 2 1/2 inches deep. Longevity of adult flies in laboratory jars was recorded as 1, 2, 3, 4 and 5 days. Four males and one female fly were identified. Dr. Sabrosky identified all Michigan rabbit examples as Cuterebra horripilum. The remaining fly which was originally sent from Pennsylvania was pupated, raised and identified as Cuterebra buccata. Because the only female fly lived one day, mode of infection studies could not be carried out. Chronologically, two adult flies, male and female, would have to emerge at nearly the same time and mate soon thereafter in order to have laboratory studies on mode of infection established through the acquisition of oviposited eggs.

10. Factors of Soil and Vegetation on Cuterebra

Results obtained from the Mason trapping projects indicated higher numbers of Cuterebra infections in areas having sandy soil. This fact may be explained by the possibility that the soil in these areas offer optimum pupation conditions. Mature larvae are better able to burrow into this soil type and thus have higher survival numbers. Inasmuch as there is better pupation results one may expect a higher concentration of pupae in these areas and subsequently greater concentration of emergent adult flies. The greater

the concentration of flies, the greater the number of infected rabbits would be in these areas. Also it was discovered that more rabbit burrows were situated in the sandy areas, perhaps allowing for optimum exposure of the hosts to the flies. Trapping records of the Michigan Conservation Department were summarized from the Rose Lake Station, Clinton County, Michigan and corroborated the results obtained at Mason. Sandy sites on the Station revealed captured cottontails with more larval infections and more larvae per number on rabbits. The records covered trapping projects dating back to 1950. Larval incidences on lower areas, such as marshes and clay soils were distinctly lower than on sandy ridge areas. Also the cottontail trapping program carried out at the Kellogg Station in 1954 supported the above contention. Mr. Aelred Geis (1955) orally stated: "the number of Cuterebra larvae were much lower in cottontails captured in low areas, such as the marshes found on the Kellogg Station, than on the sandy ridge sites." Because rabbit movement studies were being carried out on these Station areas during the summer and fall of 1954, an excellent opportunity arose for selective ecological studies of cuterebrid larvae on their hosts. However, due to their trapping procedures at Kellogg, data relative to seasonal larval incidence in cottontails was thought to be invalid.

Vegetative sites such as dense sumac bushes, willow swales and clover fields were associated with adult cuterebrid flies.

11. Effects of Larvae on Cottontails

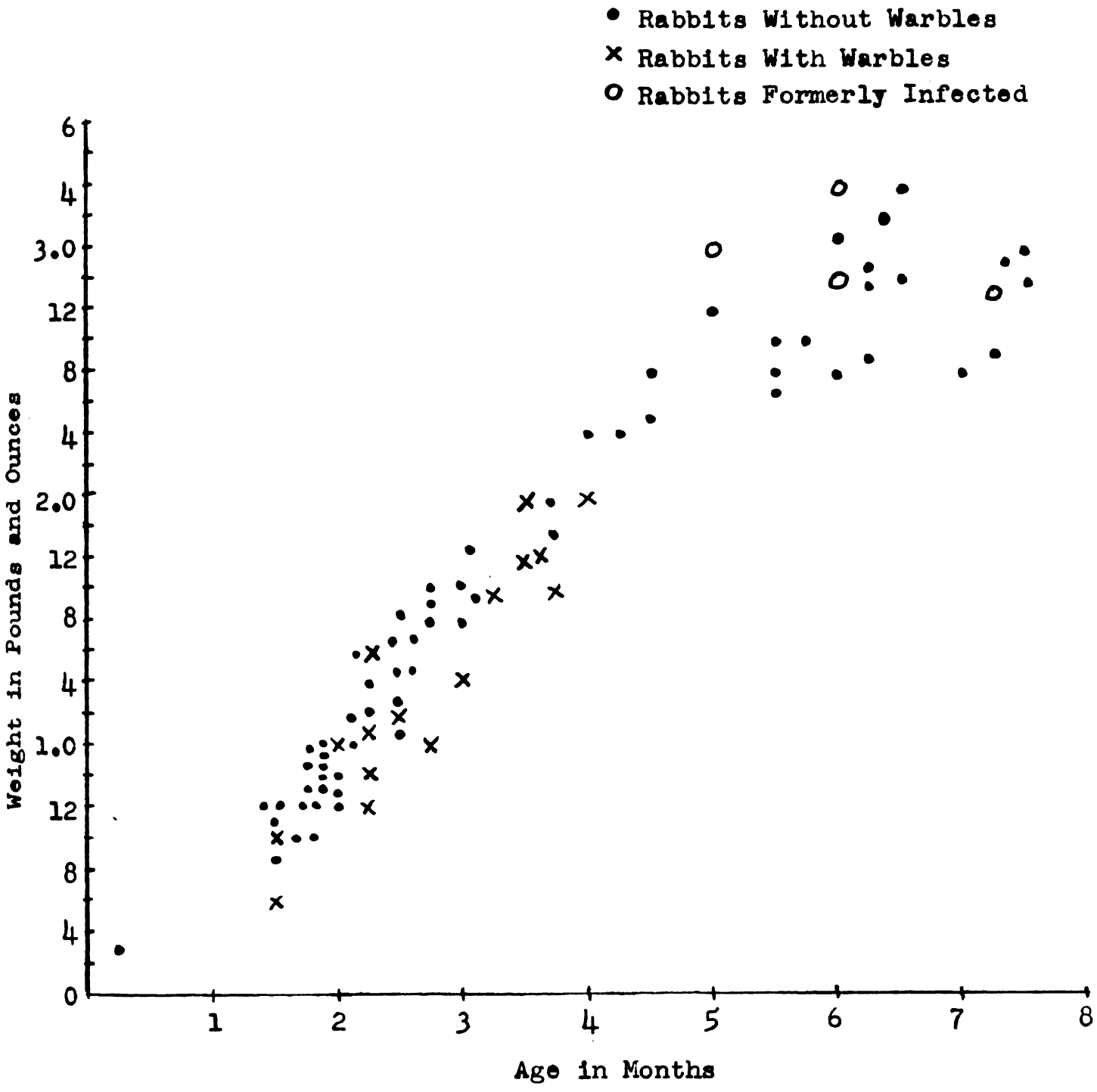
Comparing the growth curves of infected juveniles with that of adult rabbits not infected (Graph 9) demonstrates the effect on juveniles. Juvenile rabbits 2 to 3 months old, having larvae, were found, according to Geis (1953) to average about 20 percent lighter than those non-infected. Infected rabbits soon regained weight after the warbles dropped out, and averaged the same as non-infected cottontails by the fifth and sixth month.

Results of weight measurements taken on fourteen infected cottontails held in the laboratory, revealed changes in weight. This offered a good opportunity to closely check weight fluctuations. Variations in the seven infected adults were on the average 3 ounces from the original captured weight. However, the infected juveniles were observed to decrease 3 to 6 ounces from the initial laboratory weight which could prove detrimental to young who weighed 12 or 16 ounces before larval infections. Table XIII summarizes the course of a larval infection in a juvenile. This example was chosen from seven other warble infections because of its completeness and continuity of infection. The juvenile host in the earlier phase of the infection showed a debilitated condition, listlessness and lack of appetite. No diarrhea or constipation was noted and at no observed time did the rabbit lick or gnaw at the larval wound. At the beginning of observation

Graph 9

Growth Curve of Kellogg Station

Cottontails, 1951*



*From A. Geis, 1953

TABLE XIII

WEIGHT MEASUREMENTS ON A CUTEREBRA INFECTED JUVENILE COTTONTAIL CONFINED IN THE LABORATORY, 1954

Host #434-435: Juvenile Female, 1 Larva in Neck					
Days after Capture	Date	Weight (oz.)	Ounces Gained	Stage of Infection	Air Hole Diameter (mm.)
0	9-13-54	14	0	early 3rd	3
3	9-16-54	11	-3	early 3rd	7
7	9-20-54	10	-4	mid 3rd	10
11	9-24-54	11	-3	mid 3rd	12
14	9-27-54	12	-2	late 3rd	14
17	9-30-54	13	-1	late 3rd	16
19	10-2-54	16	+2	pupa	healing
23	10-6-54	20	+6	negative	scar
29	10-12-54	24	+10	negative	scar

the infection the host lost 4 ounces and its morbidity remained until the mature larva dropped from the rabbit. Once the larva left the host there was an increase in weight and this increase was retained until the tenth day after the larva dropped out, whereupon the animal, determined negative, was released into the study area.

The infected adult cottontails appeared to have more resistance to the effects of the larvae probably due to their bulk. Whereas the young cottontails which probably have just recently left their nests are more susceptible because of their lack of resistance. An autopsy on an emaciated juvenile rabbit which had been infected in the neck with three larvae revealed pulmonary congestion (pneumonia) which was thought to be a secondary effect contributory to death. The primary cause was believed to be the debilitating effects of the larvae on the young rabbit which had a dead weight of 15 ounces.

During the two-year cottontail study in southern Michigan only three cases of Cuterebra in the scrota of male cottontails were seen. These examples were from the Kellogg Station. In post-infection investigations, the resulting scar tissue from larval wounds caused severe atrophy of the scrota. Emasculation would probably ensue from the above pathology due to the lack of reproductive tissue.

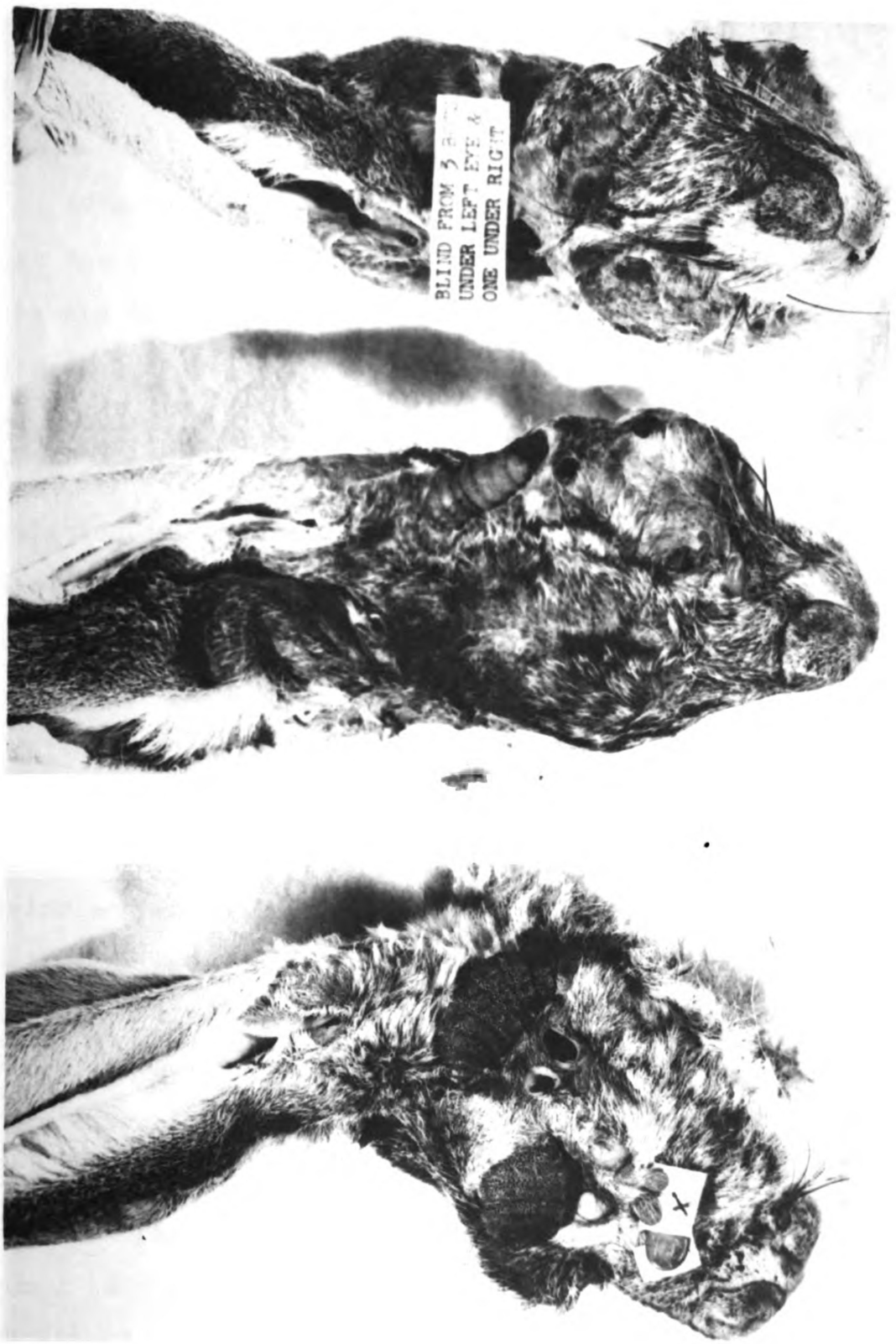
Effects of multiple infections on rabbits were observed during a cottontail hunting party at Mason, Michigan, in

November of 1954. Cottontails were being collected during the hunting season in order to observe late Cuterebra infections. An adult female rabbit which appeared to be listless when first sighted was shot. It was discovered that four larvae had recently infected the host in the neck. Three fresh larval wounds were seen minus larvae along with one late third instar. The host appeared emaciated though it was in early November and the unharvested crops on the area offered a good food supply. Upon autopsy a pulmonary congestion was again discovered. This congestion might have been one factor which slowed the animal. So indirectly the larval infections may have been contributory to the death of the rabbit by gun. Chances of winter survival in nature for an animal in this state are rather low.

Cuterebra larvae have been associated with blindness in jack rabbits of Nevada during multiple infections (Philip et al, 1955). Figure 1 shows a jack rabbit blinded from bots under left eye and one under right. There appears to be numerous bot infections in the cheek area. Source of this figure was from Dr. C. B. Philip who also studied ticks in his paper of 1955 (seen near white cards in photograph).

Fig. 1. Three heads of jack-rabbits from Nevada showing effects of bots on hosts. Head on the bottom has two third stage larvae, one in its "larval bag," the other out of it. Two air holes are seen below the free larva. Numerous warbles are found under the jaw of the middle rabbit. An early third instar is shown out of its bag. Upper rabbit was blinded because of multi-infections around the eyes.

Fig. 1



C. SYSTEMATIC STUDIES

1. Definition of Instars

After surveying 127 cuterebrid larvae it was discovered that the larval instars would have to be defined in order to separate the larvae. Larvae were sorted by external morphology according to stages prior to taxonomic determination of Cuterebra species. Criteria for larval segregation were size, shape, spine distribution, number of rows of spines, structure of the posterior spiracles, and the color of the larvae. First and second stage larvae were creamy-white in color (Figures 2 and 3). Larvae peculiar to third instars varied from light brown to black (Figure 4). The next important characteristics were the arrangement and number of rows of spines on the larval segments. Patterns for the first instar exhibited a haphazard unorganized assemblage of spines in the four anterior segments. Whereas the second stage larvae had somewhat organized anterior rows of spines on the first four segments. These two stages had spines arranged in several irregular rows that circumvented the body segments. Third stage larvae showed a distribution of spines in regular rows throughout the larval segments. Naturally in relation to size, the first instars would be the smallest. They ranged from 2 to 5 mm. in length and from 1 to 2 mm. in width. Ranges for second instars were 6 to 16 mm. in length and 2 to 6 mm.

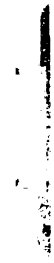


Fig. 2. First stage larva, 5 x 2 mm ., showing 12 visible segments ventrally, of which the last contains the posterior spiracles. Oral hooks are seen protruding at the right.

Fig. 2

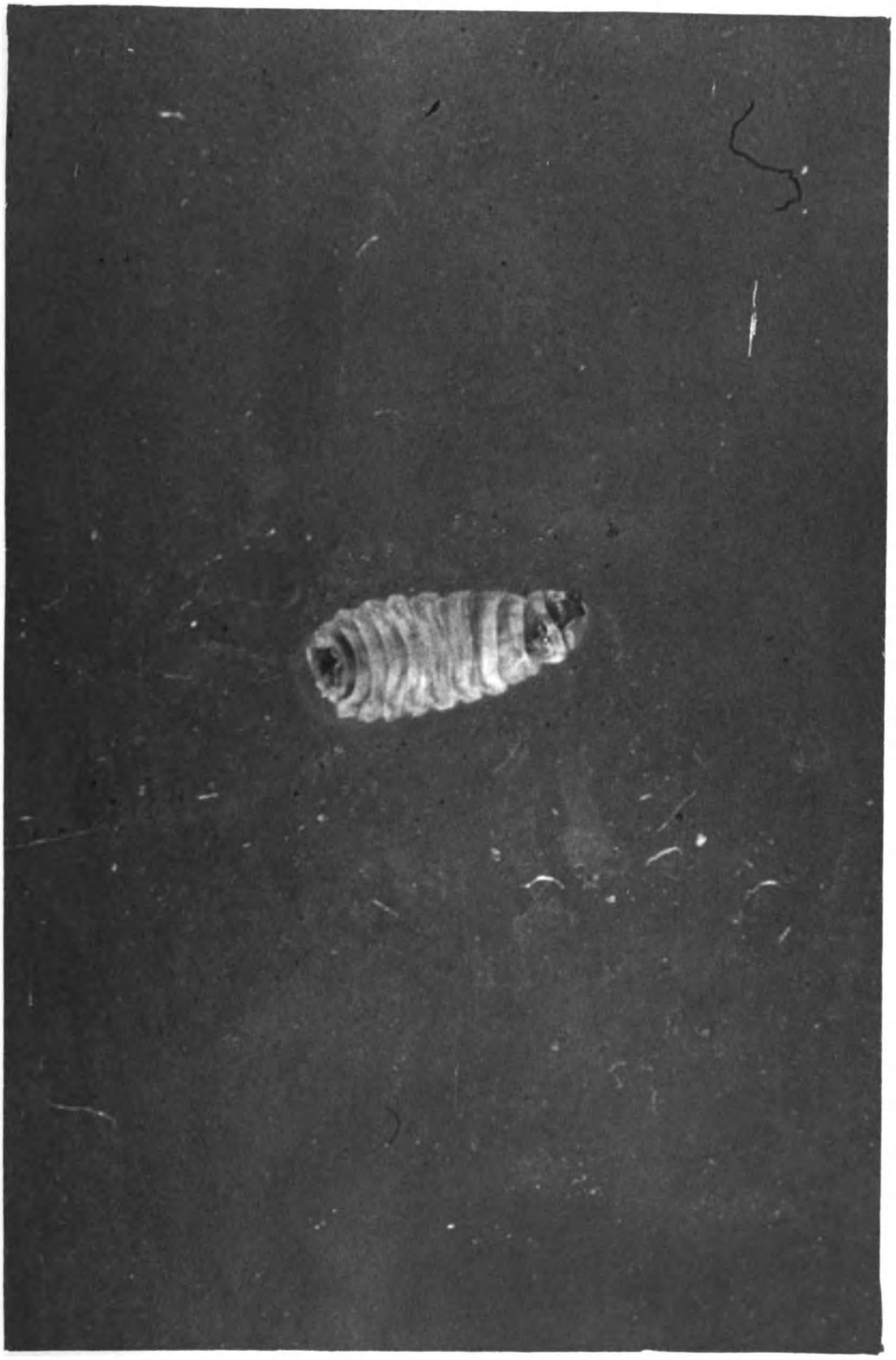


Fig. 3. A second stage larva measuring 14 x 5 mm . taken from the eyelid of a dog. The distribution and number of spines are shown on the dorso-lateral aspect beginning with the anterior segments at the upper right.

Fig. 3

Fig. 4. A third stage larva from a cottontail showing the number and distribution of spines on its dorsal surface beginning with the anterior segments at the upper right. Note the clear grooves, having microspines, one on each side of the dorsum.

Fig. 4

in width. Third stage larvae offered examples of lengths ranging from 11 to 35 mm. and widths from 6.5 to 16 mm. These measurements would vary according to Cuterebra species, and are presented here merely to relate the relative sizes of the three instars. Development of the posterior spiracles was another feature aiding in the separation of cuterebrid larvae into the various instars. Posterior spiracles peculiar to third instars (Figure 5) show each plate divided in three parts and perforated by numerous sinuous spiracular openings. Spiracular plates for young Cuterebra larvae were not clearly defined and may have two sinuous spiracular openings (Figure 6).

2. Hosts Studied

Cuterebrid larval material collected from various sources was studied separately according to the hosts they infected. This was done after the larvae were differentiated to various instars. The host examples included small wild mammals whose scientific names and general location are as follows: white-footed field mouse, Peromyscus leucopus, Michigan; Sylvilagus floridanus mearnsii, cottontail of Michigan, Pennsylvania and Wisconsin; Sylvilagus floridanus mallurus, cottontail found in New Jersey; and the jack rabbit, Lepus californicus deserticola of the state of Nevada. Larvae from domestic

Fig. 5. Posterior spiracular plates and spiracles of a mature C. fontinella larva taken from a white-footed mouse.

Fig. 5

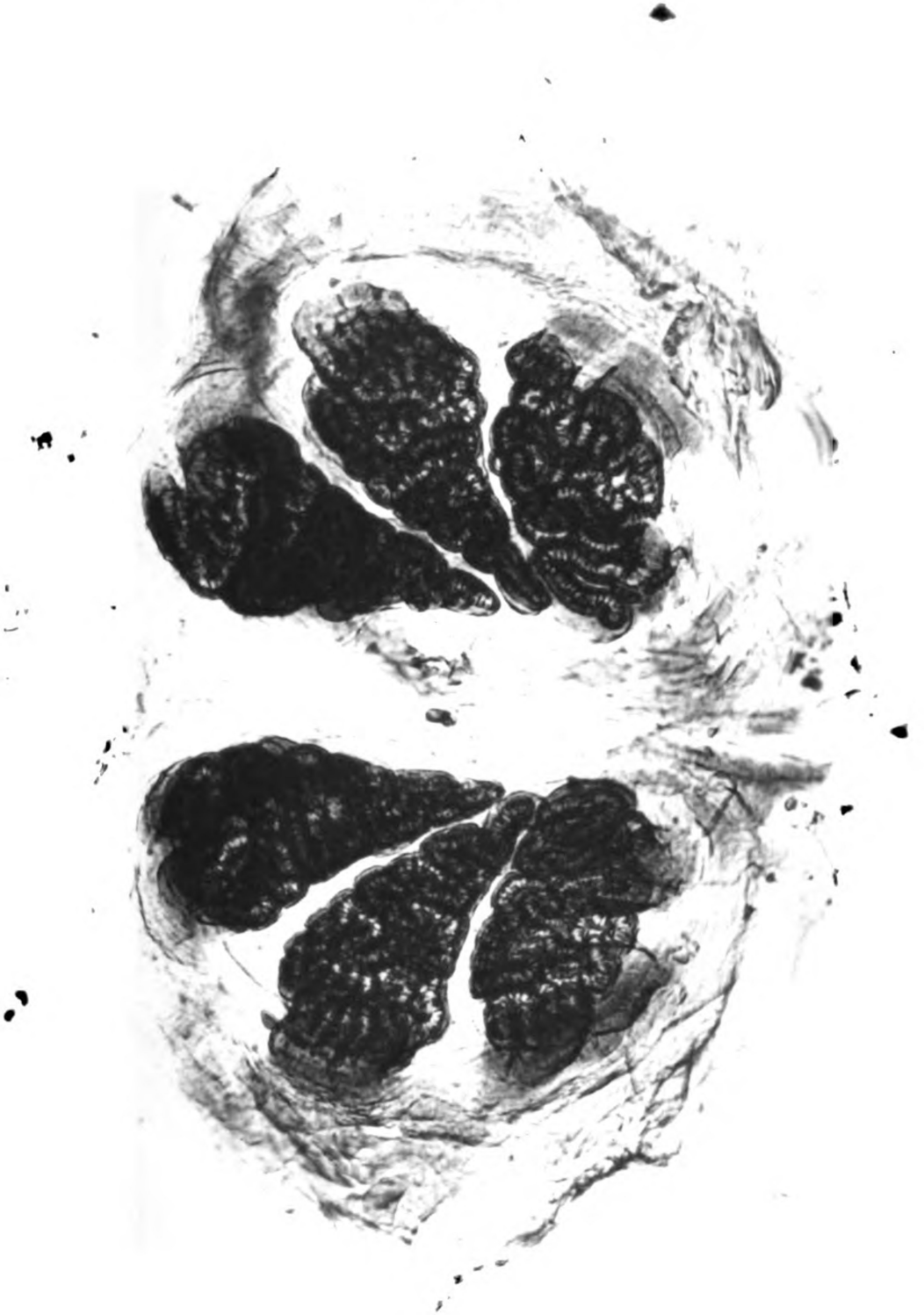


Fig. 6. Highly magnified posterior spiracles of second stage C. fontinella larva taken from a white-footed mouse.

Fig. 6

mammals were obtained from the dog, Canis familiaris, and the cat, Felis domestica, whose locations were listed as Wisconsin, Ohio, Michigan and Illinois.

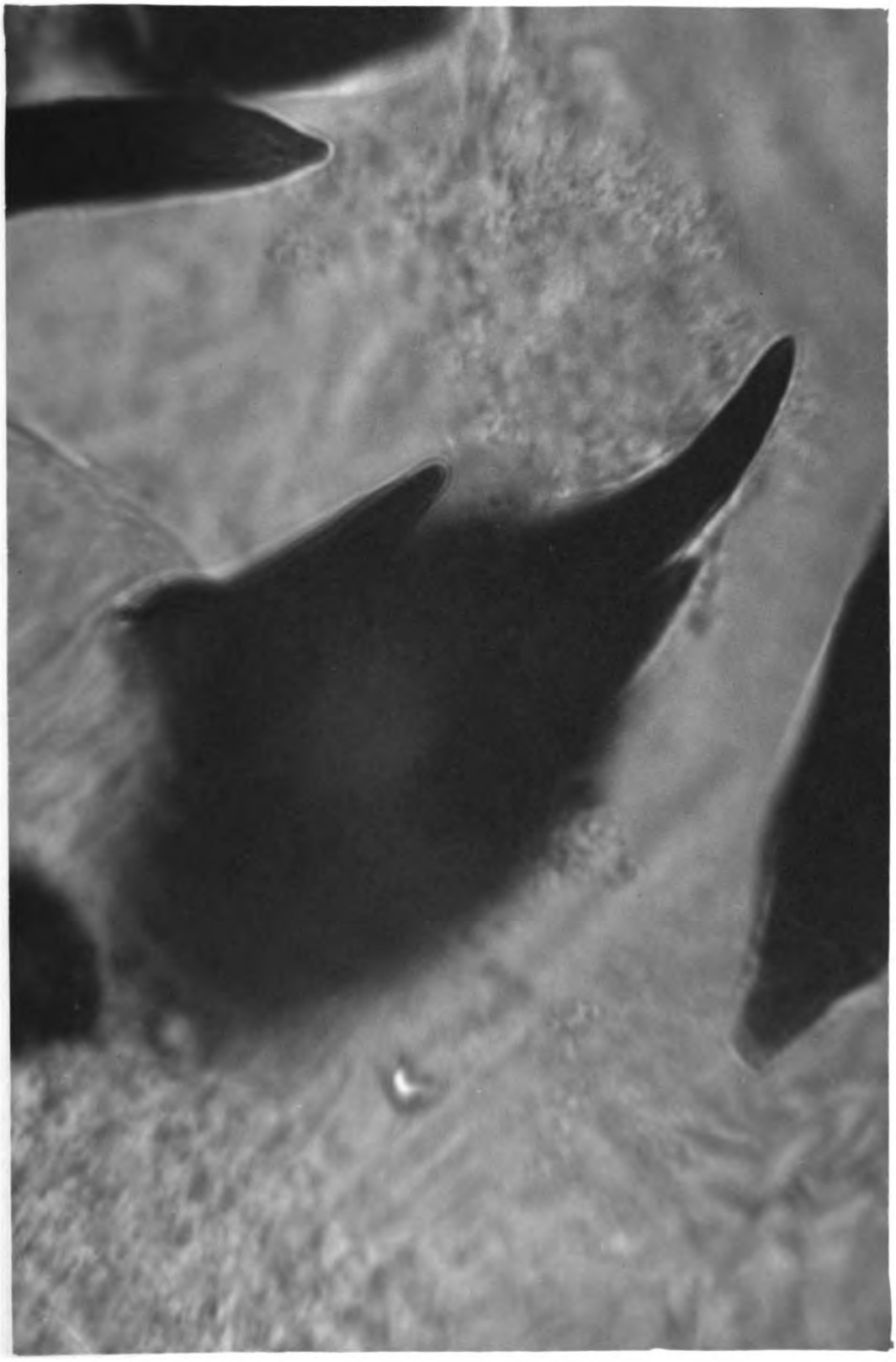
3. Cuterebra in Felis domestica

a. C. horripilum

First stage. Five examples of this instar were primarily studied in respect to arrangement and number of rows of spines found dorsally and ventrally on the larval segments. Average length and width of the larvae were 4.75 mm. and 1.75 mm. respectively. These measurements were obtained from ranges of 3 to 6 mm. for the length and of 1 to 2 mm. for the width. All representative instars were white in color. However, the shape of spines varied in one specimen. Four of the five larvae had predominantly single pointed spines along with a few multipointed spines (Figure 7). The remaining larva had only single pointed spines. However, the latter example was an early first instar and believed to be incomplete for that stage in its spine development. Thus, these larvae may be all from the same species, but not quite in the same phase of the first stage. Posterior spiracles added evidence to this question by comparing their structures. Before the investigation of this question, the pattern of the spines per segment will be presented below.

Fig. 7. Multipointed spines of a C. horripilum first stage larva taken from Felis domestica.

Fig. 7



Cephalic segment. Small and usually partly retracted. Anterior lobes bear small sensory tubercles. Group of spines is found ventrally near the mouth.

Segment 2. Anteriorly 4 rows of caudad pointed spines. No row of spines posteriorly on segment.

Segment 3. Anteriorly 4-5 rows of caudad pointed spines. No row of spines posteriorly on segment.

Segment 4. Anteriorly 4-5 rows of caudad pointed spines. No row of spines posteriorly.

Segment 5. Anteriorly 4-5 rows of caudad pointed spines No row of spines posteriorly.

Segment 6. Anteriorly 4-5 rows of caudad pointed spines. Posteriorly 1 row of cephalad pointed spines.

Segment 7. Anteriorly 3-4 rows of caudad pointed spines. Posteriorly 1 row of cephalad pointed spines.

Segment 8. Anteriorly 3-4 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 9. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 10. Anteriorly 1 row of caudad pointed spines. Posteriorly 6-8 rows of cephalad pointed spines.

Segment 11. Anteriorly 1 row of caudad pointed spines. Posteriorly 4-5 rows of cephalad pointed spines.

Segment 12. More or less truncate behind. Rounded anal lobes on each side of anus. Posterior spiracles in center of segment at the end.

Segmental boundaries of the first four segments were difficult to recognize in preserved specimens due to the intermingling of the posterior spines of the preceding segment into the anterior spines of the following segment. In general, the spines appeared, at first glance, to have a haphazard pattern in direction. No spines were apparent between the anterior and posterior spines of a segment. The length and width of the largest spines ranged from .12 to .14 mm. and .043 to .072 mm. respectively. Patterns of the posterior spiracles in all five examples were similar to that seen in Figure 8. Spiracular plates which measured .48 mm. wide and .36 mm. long were not organized in any defined pattern and showed long moderately convoluted spiracles which were broken into segments. Each spiracle ended in two slits and measured .22 and .11 mm. for the length and width.

On the basis of two pointed spines which Knipling and Brody (1940) stated were occasionally seen in C. horripilum, it is believed that these first instar larvae from the cat were Cuterebra horripilum. Two-pointed spines were occasionally seen on the larval segments, especially in the anterior rows of the first four segments and in the twelfth segment.

Oral mouthparts for this instar extended back to the anterior margin of the third segment. Morphology of the cephalopharyngeal skeleton is reflected in Figure 9. The oral skeletons measured .72 mm. for the length and .32 mm.

Fig. 8. Posterior spiracular plates of a first stage larva, C. horripilum, obtained from Felis domestica.

Fig. 8

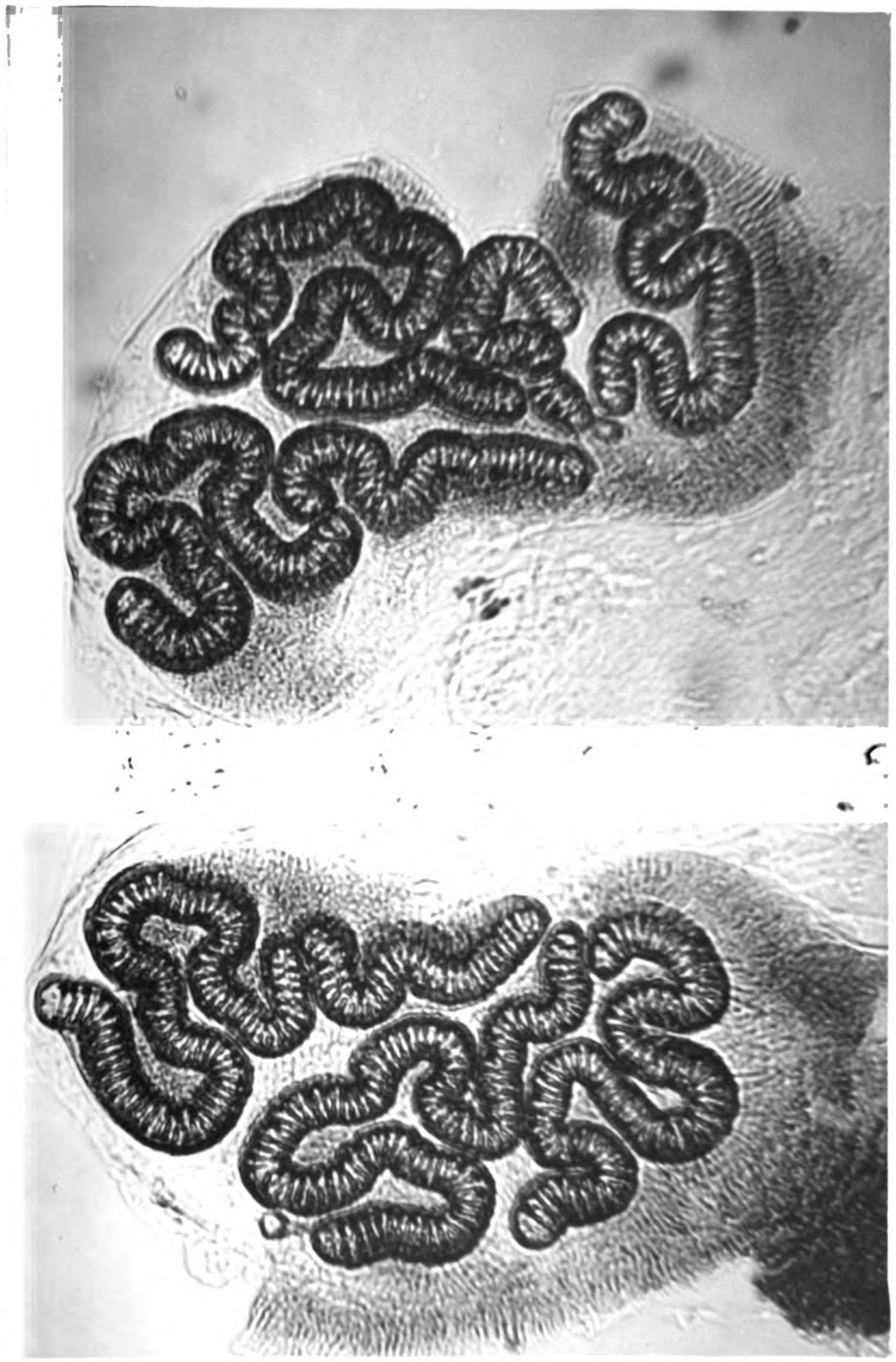


Fig. 9. The left side of a cephalopharyngeal apparatus, dissected from a first instar of C. horripilum, with Felis domestica as the host. Note the foramen in the oral hook sclerite.

Fig. 9

for the width. Determination of the length was arrived at by measuring the oral apparatus from the oral hook to the end of the dorsal cornu. For the width, the distance between the dorsal and ventral cornu was the measurement. This procedure was carried throughout the measurements of the oral skeletons.

Second stage. Twelve larval representatives were collected for taxonomic investigations. They were white in color and averaged 11.0 and 4.5 mm. in length and width. The shape of the spines was predominantly single pointed, although two-pointed spines were occasionally seen during the screening of the segments under a stereoscope. Larger spines were .35 mm. long and .16 mm. wide. Segment 7 revealed 5 to 7 rows of spines, and the remaining ventral segments offered an arrangement and number of spines similar to that situation existing in the second instars from Peromyscus leucopus. The following is a presentation of the pattern and number of spines present on the dorsal surface of second instars collected from cats. Segment 1 was usually retracted and small with two anterior lobes having small sensory tubercles. Ventrally, near the mouth a group of spines were found. Segment 12 was truncate behind and contained the anus. The posterior spiracles are situated at the end and in the center of the segment.

Segment 2. Anteriorly 4-5 rows of caudad pointed spines. No spines posteriorly.

Segment 3. Anteriorly 4-5 rows of caudad pointed spines. No spines posteriorly.

Segment 4. Anteriorly 4-5 rows of caudad pointed spines. No spines posteriorly.

Segment 5. Anteriorly 4-6 rows of caudad pointed spines. Posteriorly 1 row of cephalad pointed spines.

Segment 6. Anteriorly 4-6 rows of caudad pointed spines. Posteriorly 2 rows of cephalad pointed spines.

Segment 7. Anteriorly 4 rows of caudad pointed spines. Posteriorly 4-5 rows of cephalad pointed spines.

Segment 8. Anteriorly 4 rows of caudad pointed spines. Posteriorly 4-5 rows of cephalad pointed spines.

Segment 9. Anteriorly 3-4 rows of caudad pointed spines. Posteriorly 4-5 rows of cephalad pointed spines.

Segment 10. No row of spines either anteriorly or posteriorly. Few scattered spines posteriorly.

Segment 11. Anteriorly few microscpines. Posteriorly 8-10 rows of cephalad pointed spines.

However, the size and general shape of the dorsal spines varied in the two host examples. The oral skeleton for this stage from the cat was .86 mm. long and .43 mm. wide (Figure 10.). Spines found dorsally on segment 11 were generally the

Fig. 10. The right side of a cephalopharyngeal apparatus dissected from a second stage larva of C. horripilum. Foramen is seen in the oral hook sclerite.

Fig. 10

same as the situation on the ventral aspect. Segment 12 had spines on its anterior segments gradually reduced, whereas the posterior half of the segment was well armed.

There were three definite slits in the posterior spiracles of this instar and the arrangement of the spiracles appeared to be approaching a fixed structure typical of third instars (Figure 11). Length and width of the spiracles were .65 and .42 mm. respectively. The spiracular plates were .24 mm. wide and .42 mm. long. No buttons were present in any of the twelve instar examples. Cuterebra horripilum appeared to be the species for these larvae from the cat.

Third stage. Approximately eighteen larvae were taxonomically studied in this group. Third stage larvae reflected color variances from light brown to black. A few two pointed spines were discovered on the larval segments. The larval examples had an average length and width of 15.0 and 6.25 mm. accordingly. Ranges of the larvae extended from 11 to 20 mm. in length and 6 to 11 mm. in width which were interpreted as early and mid third instar larval examples. Larger spines had a length range of .33 to .42 mm. and widths ranging from .28 to .34 mm. respectively. Number of rows of spines in segment 7 was 12 to 15 which was the average condition for all segments. Also in segment 7 mature spines were seen of the first 4 to 5 rows anteriorly and posteriorly with haxagonal untipped spines in between these rows. The above

Fig. 11. Posterior spiracular plates and spiracles of a second stage larva of C. horripilum obtained from Felis domestica.

Fig. 11

spine pattern was typical of early third stage larval segments. Tips of these spines were either light brown or black depending on degree of larval maturity.

Posterior spiracles for these two-pointed spine examples (Figure 12) measured 1.6 to 1.3 mm. in average length and width. The spiracular plates revealed a diffused pattern of spiracles not clearly defined by two slits in each plate. Spiracular plates were 1.4 mm. long and .58 mm. wide. Semblance of a button may be seen in the mid-line of the spiracle. Figure 13 revealed the typical morphology of the oral skeleton which gave an average length and width of 2.2 and 1.08 mm. respectively. The species of Cuterebra determined for these two-pointed spine larvae was Cuterebra horripilum.

b. C. buccata

Third stage. Fifteen larvae with single pointed spines ranged in length from 15 to 30 mm. and in width 8 to 13 mm. The minimum points of these ranges represented early third stages and the maximum points were of the mature larvae. Larger spines of mature larvae were .38 to .45 mm. long and .28 to .32 mm. wide. The number of spinal rows in segment 7 were 12 to 15. Of significant difference was the structure of the posterior spiracles which showed two slits clearly separating three bundles of spiracles in each spiracular plate (Figure 14). Average lengths and widths of the posterior

Fig. 12. Posterior spiracular plates and spiracles of a third stage larva of C. horripilum obtained from a cat. Note the semblance of buttons in the centre of the plates.

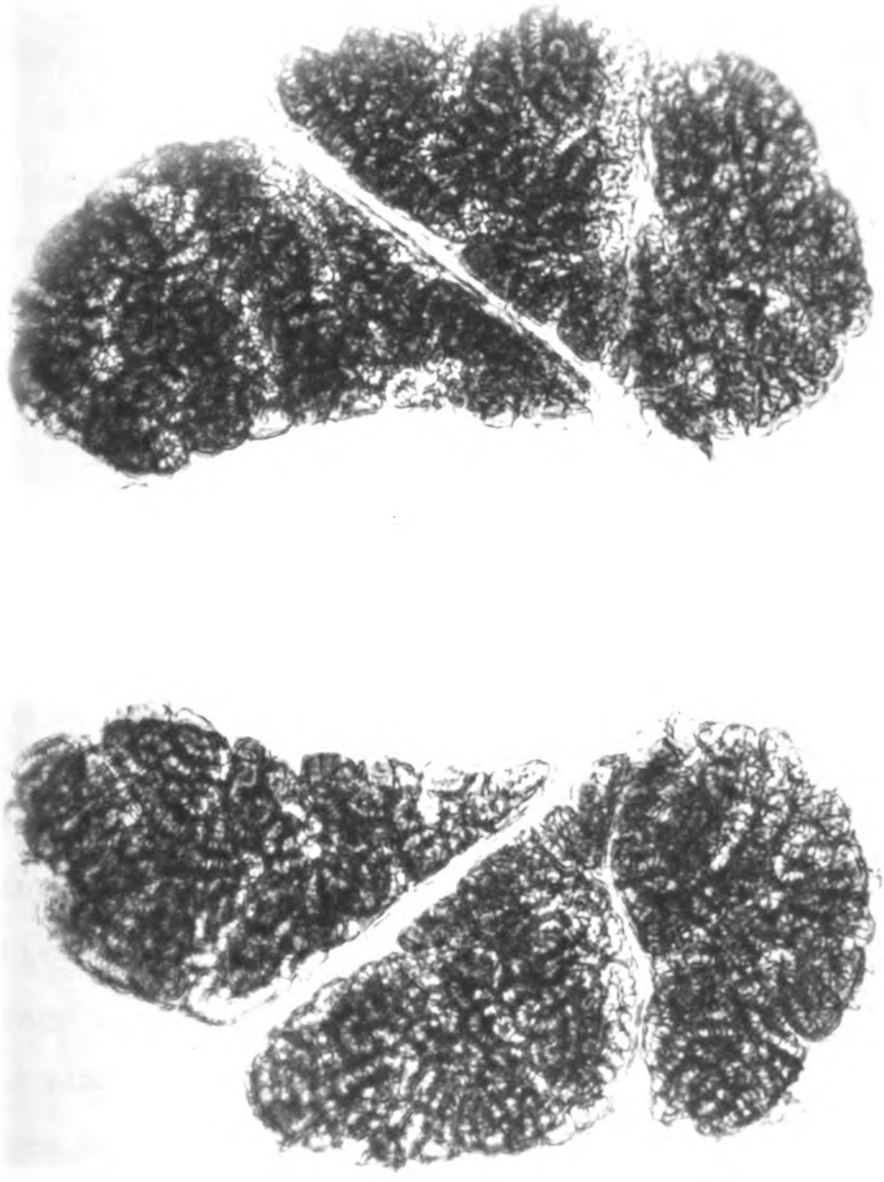
Fig. 12

Fig. 13. The right side of a cephalopharyngeal apparatus of a C. horripilum third stage larva obtained from a cat. Note foramen in the slender oral hook sclerite.

Fig. 13

Fig. 14. Posterior spiracular plates and spiracles of a third stage larva of C. buccata obtained from a cat. Note narrow width of the convoluted chains.

Fig. 14



spiracles were 1.04 and 1.34 mm. respectively. Spiracular plates measured 1.00 mm. in length and .50 mm. in width. What might be interpreted as a button was vaugely seen in the middle portion of the posterior spiracles.

The oral skeleton was found to be comparatively smaller than C. horripilum, measuring 2.04 and .75 mm. in length and width (Figure 15). All measurements taken on larval examples are averages and may be interpreted as such. For these larvae Cuterebra buccata Fabricius was the determined species.

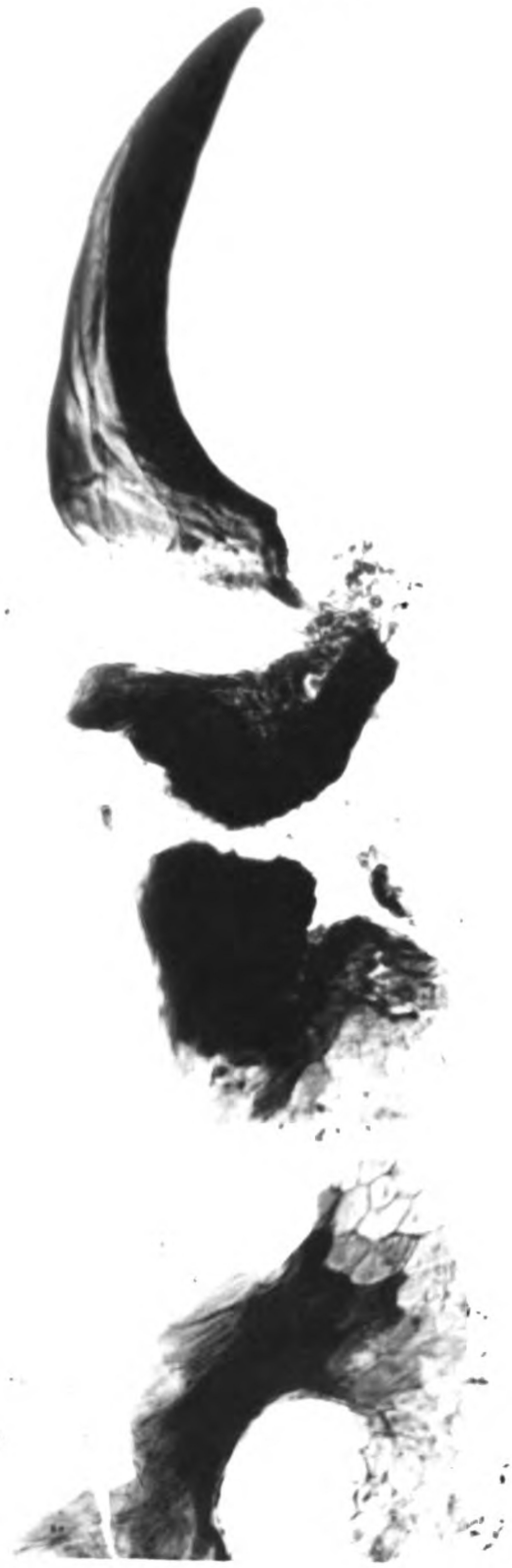
4. Cuterebra in Canis familiaris

a. C. buccata

Second stage. Two larval specimens of this instar were obtained from the dog. These were studied for taxonomic differences among the dog, cat, and rabbit species. The white larvae gave an average length and width of 14 and 5 mm. respectively. Spines were slender and single pointed with an average length and width of the larger spines of .32 and .17 mm. accordingly. The larvae had a pattern and number of spines similar to that found in the cat, but having their spines slightly smaller and more delicate in appearance than the feline examples. Measurements taken on the oral mouthparts were smaller compared to those obtained for cats. The mouthparts were .72 mm. long and .36 mm. wide (Figure 16).

Fig. 15. The left side of a cephalopharyngeal apparatus of a C. buccata third stage larva obtained from a cat.

Fig. 15



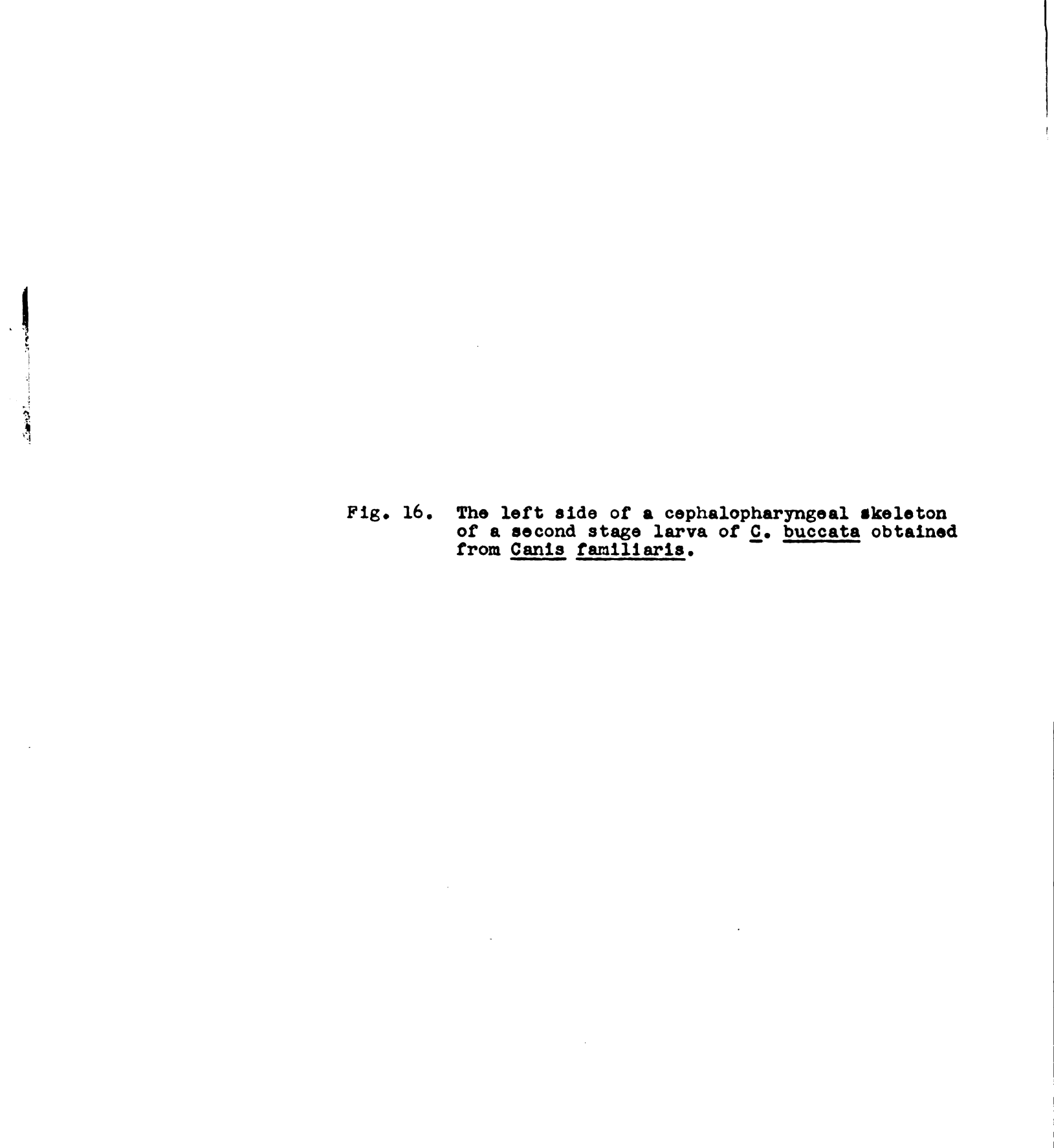


Fig. 16. The left side of a cephalopharyngeal skeleton of a second stage larva of C. buccata obtained from Canis familiaris.

Fig. 16

Also the posterior spiracles (Figure 17) were smaller than the cat sample, measuring .43 and .32 mm. for the length and width. By comparison of the posterior spiracles taken from the cat and the dog Cuterebra, it appeared that the spiracles on the larvae from the dog were not fully developed to final structure and were in the process of maturing so as to array themselves in similar morphology to spiracles from the cat Cuterebra larvae. No buttons were discernable.

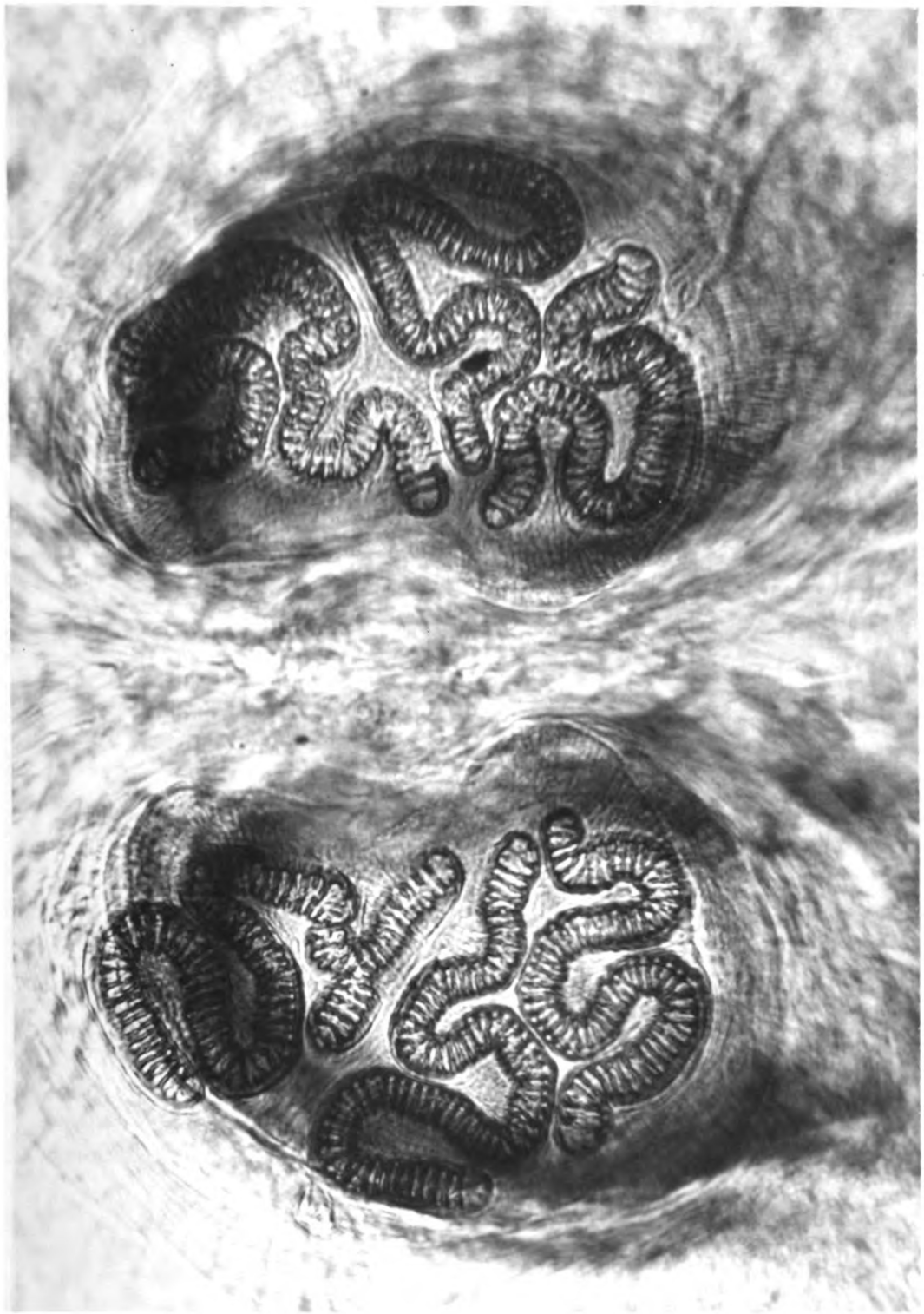
On the bases of single pointed long delicate spines smaller oral skeletons and posterior spiracles, these two second instar examples from the dog are presented as Cuterebra buccata. The location of infection for these larvae was in the neck and records do not show the sexes of the dogs, which might explain the site in the neck and not in the scrotum which is usually associated with this cuterebrid species.

5. Cuterebra in Lepus californicus deserticola

Second stage. Only one larval specimen of this instar was obtained from Dr. Jellison. This larva of Nevada jackrabbits was white in color and measured 14 and 6 mm. in length and width. Measurements of the larger spines gave a length and width of .29 and .17 mm. respectively. Survey of the spines showed the shape of these spines to be single and two pointed. Numerous multi-pointed spines were seen in segments 2 to 4 and 11. The spine pattern and number

Fig. 17. Posterior spiracular plates and spiracles of a second stage larva of C. buccata obtained from a dog.

Fig. 17



of rows was essentially the same as those of the cat, dog and rabbit arrangements, but less rows were present. Because of the unusual host and the geographic location of the larva, the scheme of dorsal spines is presented below for comparison with those of the cat, dog and rabbit. Segments 1 and 12 again were similar to previous larval examples of the cat, dog, and rabbit. On the ventral surface there were no rows of spines posteriorly in segments 2 to 10.

Segment 2. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly no spines.

Segment 3. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly no spines.

Segment 4. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly no spines.

Segment 5. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1 row of cephalad pointed spines.

Segment 6. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 7. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 8. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 9. Anteriorly 2-3 rows of caudad pointed spines. Posteriorly 1-2 rows of cephalad pointed spines.

Segment 10. No spines either anteriorly or posteriorly.

Segment 11. No spines anteriorly.

Posteriorly 6-8 rows of cephalad pointed spines.

Taxonomic differences were noted in this larval example. The first apparent difference, besides the shape of the spines, was the structure of the posterior spiracle (Figure 18). Four vertically arranged spiracles having an average length and width of .14 and .03 mm. were observed. Individual spiracular plates were .15 mm. long and .09 mm. wide. Total length and width of the posterior spiracles was .26 and .15 mm. respectively. No buttons were present. Comparatively, this posterior spiracle was quite small and structurally different than those of Cuterebra horripilum and C. buccata. The morphology of the oral mouthparts (Figure 19) showed a difference in the area of the oral hook and appeared to be stubbier. However, the measurements of the oral skeleton were .72 mm. long and .29 mm. wide.

Proposed cuterebrid species for this larva was rather difficult inasmuch as the literature is rare on these rabbit larvae. Yet, the posterior spiracles resemble those peculiar to the early instars of C. fontinella. Philip et al. (1955) state that C. ruficrus and C. jellisoni have been found on Nevada jack rabbits.

Fig. 18. Posterior spiracular plates and spiracles of a second stage larva obtained from Lepus californicus deserticola.

Fig. 18

Fig. 19. The right side of a cephalopharyngeal apparatus of a second stage larva obtained from Lepus californicus deserticola. Note stubby oral hook sclerite.

Fig. 19

Third stage. Eighteen larvae which were collected from Nevada jack rabbits were sent by Dr. Jellison for study. These larvae averaged 25.4 and 11.2 mm. in length and width. Averages were taken from larvae ranging 19 to 35 mm. in length and 9 to 16 mm. in width. The shape of the spines on these larval segments revealed single, double and triple points. Seven larvae exhibited only the single oblique cone-shaped spines. These long tipped spines gave ranges in length and width of .38 to .58 mm. and .32 to .54 mm. respectively. The number of rows of spines on these specimens were between 15 and 20. A typical larval segment such as number 7 would have the first 5 to 6 rows anteriorly and the last 5 to 6 rows posteriorly with single long obliquely slanted spines and the intermediate spines were short and stubby. Segment 11 had short sharply pointed spines with black tips. The last 5 to 6 rows of this segment had spines which were orange in color with black tips.

Larvae with oblique cone shaped multipointed spines were essentially the same in length and width as the single pointed larger spines and similar in rows of spines in segment 7. However, segment 11 was not orange in color. Multipointed spines were found mostly on the first three anterior and posterior rows. Intermediate spines were seen forming two-pointed spines. More multipointed spines were seen dorsally than ventrally on the larval segments. Spines

surrounding the posterior spiracle in segment 12 also revealed multipointed spines.

After examining the posterior spiracles of all the jack rabbit larvae, it was discovered that regardless of whether the larval spines were single or multipointed the shape of the spiracles was similar (Figure 20). Scrutinizing this spiracular example, it was compared with that of C. buccata. However, the spiracles of buccata were long convoluted unbroken in character whereas the spiracles of these Nevada cuterebrid larvae were thicker in diameter and broken up into a "macaroni" pattern. The macaroni pattern of these spiracles was the same in the two types of larvae under consideration, that is, the single and multipointed spine types. Measurements on the posterior spiracles were .65 to .80 mm. wide and 1.01 to 1.20 mm. long respectively. Oral mouthparts of the two larval types were also found similar if not identical in morphology and approximate measurements (Figure 21). Lengths and widths ranged between 2.30 to 2.44 mm. and 1.00 to 1.08 mm. respectively. Two species, namely, C. ruficrus and C. jellisoni were recorded in the literature from Nevada jack rabbits (Philip et al., 1955). Further mention was made in this reference that both species have been reared from jack rabbits. In order to definitely identify these it would be necessary to have the puparial cases related to the Cuterebra species. At this point one could only present the larval taxonomic findings sine confirmation of Cuterebra species.

Fig. 20. Posterior spiracular plates and spiracles of a third stage larva obtained from Lepus californicus deserticola.

Fig. 20

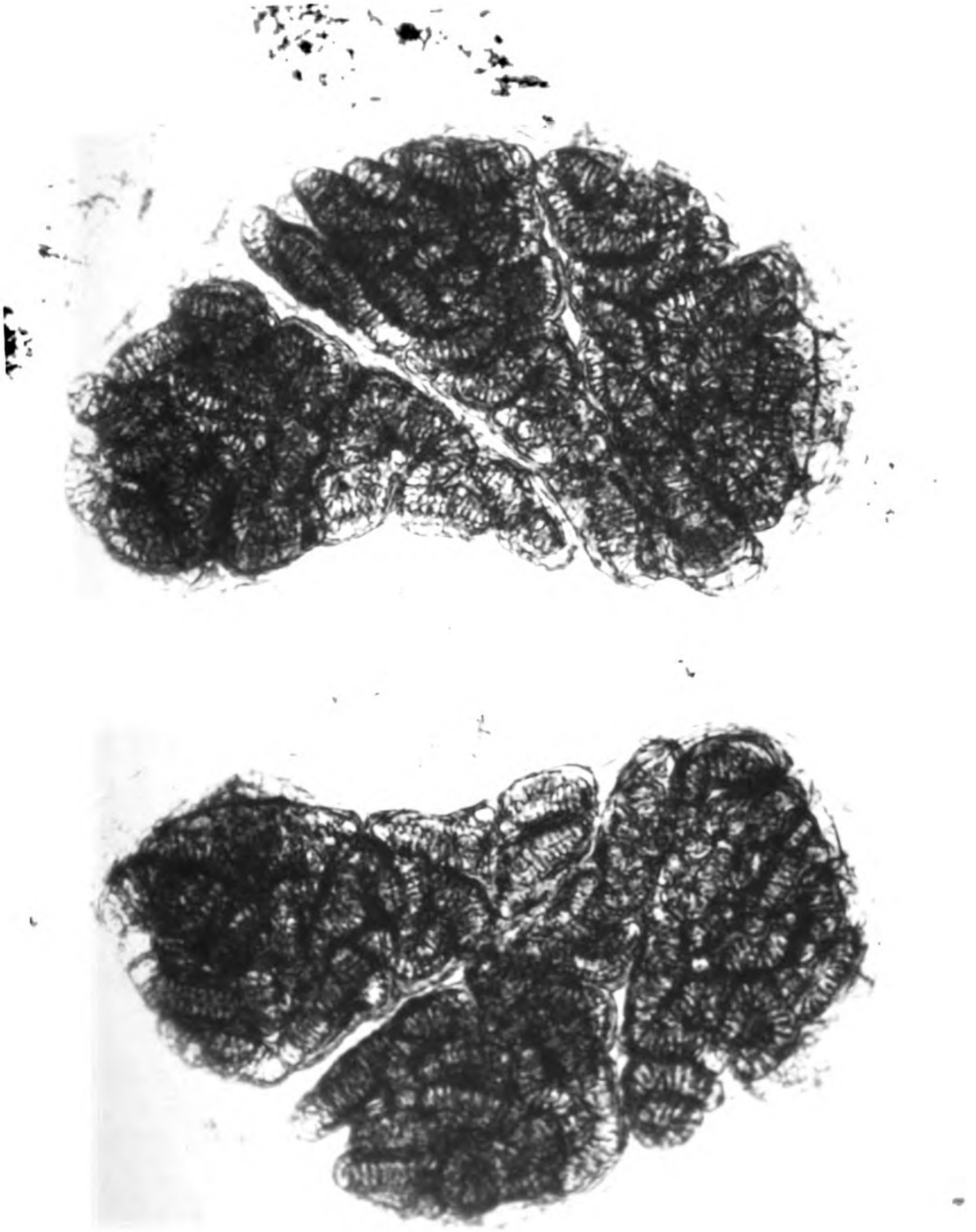


Fig. 21. The left side of a cephalopharyngeal apparatus of a third stage larva obtained from Lepus californicus deserticola. Foramen in the oral hook sclerite was not discernible.

Fig. 21

Puparium. With regard to the Cuterebra larval species described from Nevada rabbits, no puparia were obtained in order to taxonomically separate the species, namely C. ruficrus or C. jellisoni. No relationship could be made to the larval descriptions rendered. This adult fly-pupa relation will have to be accomplished before positive identification of these larvae can be made.

6. Cuterebra in Peromyscus leucopus

a. C. fontinella

Second stage. Three specimens were collected and studied according to the taxonomic characteristics of the early instars. Length and width averages for these instars were 9.5 to 4.3 mm. respectively. These averages were taken from ranges of 9 to 10 mm. for length and 4 to 5 mm. for width. All larvae were white in color. Long tipped spines, found on the segments, exhibited 1 to 3 points upon examination, especially in segment number eleven. These spines had an average length and width of .09 and .03 mm. The pattern of spines peculiar to these instars are described below for each segment.

Segments 1 and 12 were generally similar to the early instars previously described, except for a slight increase in the number of spines on these segments.

Segment 2. Anteriorly 3-4 rows of caudad pointed spines.
First 2 rows have largest spines.

Segment 3. Anteriorly 3-4 rows of caudad pointed spines.
No posterior spines.

Segment 4. Anteriorly 3-4 rows of caudad pointed spines.
No posterior spines.

Segment 5. Anteriorly 4-5 rows of caudad pointed spines.
No posterior spines.

Segment 6. Anteriorly 4-5 rows of caudad pointed spines.
Posteriorly 1-2 rows of cephalad pointed spines.

Segment 7. Anteriorly 4-5 rows of caudad pointed spines.
Posteriorly 1-2 rows of cephalad pointed spines.

Segment 8. Anteriorly 4-5 rows of caudad pointed spines.
Posteriorly 2-3 rows of cephalad pointed spines.

Segment 9. Anteriorly 4-5 rows of caudad pointed spines.
Posteriorly 2-3 rows of cephalad pointed spines.

Segment 10. No spines anteriorly.
Posteriorly 3-4 rows of cephalad pointed spines.

Segment 11. No spines anteriorly.
Posteriorly 4-5 rows of cephalad pointed spines.

Measurements of the larger spines were .27 mm. long and .16 mm. wide. Rows of spines taken arbitrarily on segment 7 were between 5 and 7 in number. This procedure was undertaken for every larval stage and host. The above three specimens were located in the neck of the host. No examples of larvae

in the scrotum were obtained for this stage. The cephalopharyngeal apparatus measured 1.22 mm. long and .65 mm. wide, but was otherwise too badly damaged for morphological comparative study. However, embedded in the larvae, an additional oral skeleton was found which was smaller in size resembling a first stage larval oral apparatus (Figure 22). The latter example was more intact and measured .48 mm. long and .17 mm. wide.

Structure of the posterior spiracles (Figure 5) showed vertical spiracles measuring .10 mm. long and .028 mm. wide. Posterior spiracular plates measured .17 mm. wide and .14 mm. long. There was no evidence of a button in the posterior spiracles. The cuterebrid species peculiar to these three similar second instars was Cuterebra fontinella Clark.

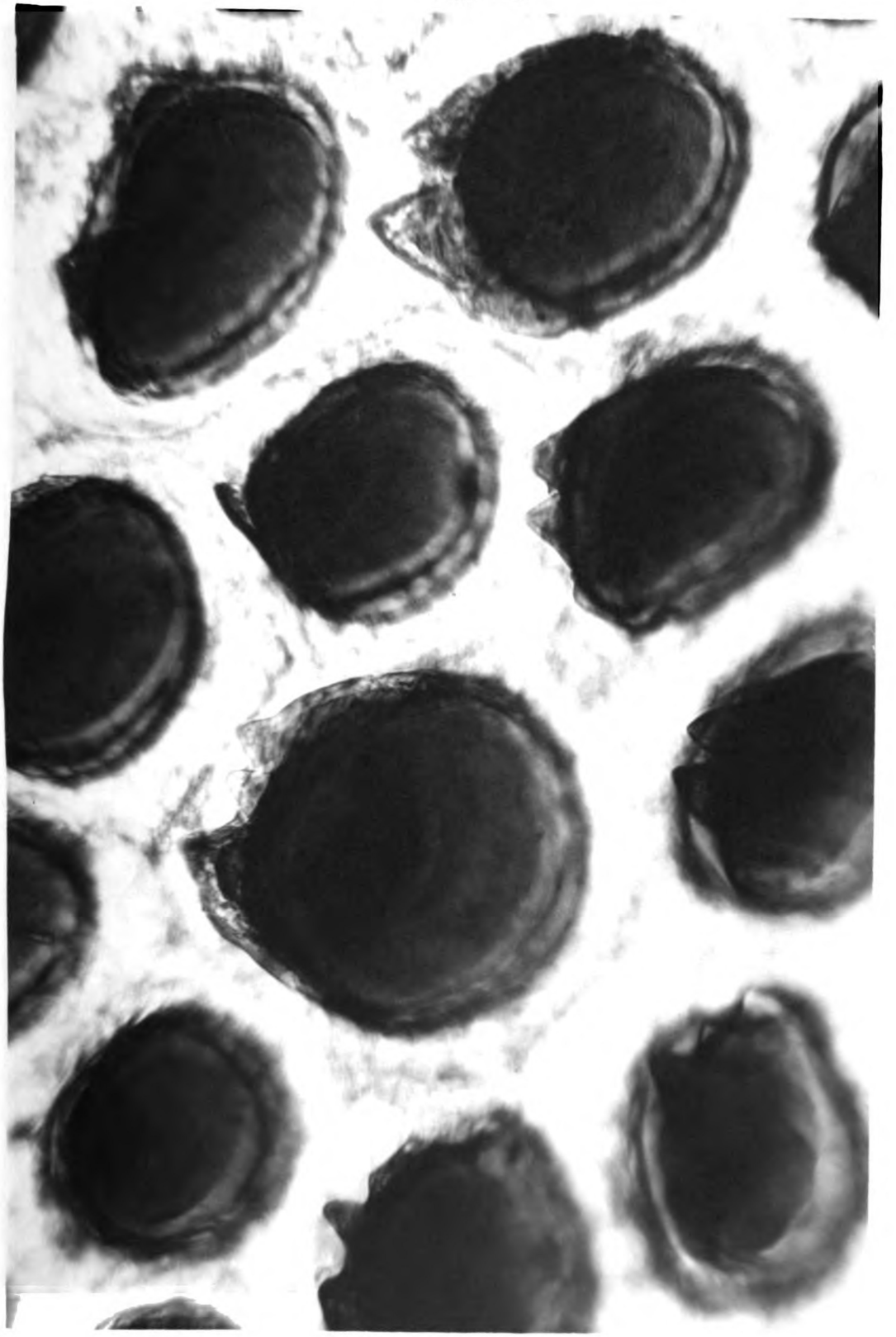
Third stage. Five larval examples of third instars were collected for the systematic study. Locations of these larvae at time of the collection were in the neck and in the scrota. The color of the larvae varied from light brown to brownish black. The average length and width of these third stage larvae was 14.6 and 7.9 mm. These measurements were taken from larvae offering ranges of 10 to 22 mm. in length and 6 to 11 mm. in width. Ranges for the length and width of the larger spines were .28 to .32 mm. and .19 to .21 mm. respectively. One to six points were discovered on the short stubby spines (Figure 23) and were frequently seen in the larval segments. In segment 7 the rows of spines were found to be

Fig. 22. The cephalopharyngeal apparatus of a first stage larva of C. fontinella, obtained from a white-footed mouse. Note the foramen in the mandibular hook sclerite.

Fig. 22

Fig. 23. Multi-pointed spines of a third stage larva of C. fontinella obtained from a white-footed mouse.

Fig. 23



from 15 to 20. The arrangement and number of rows of multispines per segment is described below.

Segment 1. No multispines present.

Segment 2. Anteriorly, multispines present in first 2-3 rows.
Posteriorly, multispines in last row.

Segment 3. Same as 2.

Segment 4. Same as 2.

Segment 5. Same as 2.

Segment 6. Anteriorly in first 5 rows.
Posteriorly last 2 rows.

Segment 7. Anteriorly in first 5-6 rows.
Posteriorly last 2-3 rows.

Segment 8. Anteriorly in first 6 rows.
Posteriorly in last 3 rows.

Segment 9. Anteriorly in first 6-7 rows.
Posteriorly in last 2-3 rows.

Segment 10. Anteriorly in first 6-7 rows.
Posteriorly in last 2-3 rows.

Segment 11. Anteriorly in first 6-7 rows.
Posteriorly in last 3 rows.

Segment 12. Multispines scattered throughout segment.

It was noted upon microscopical examination of the spines that the larvae from the white-footed mouse were red in color, especially in segment 9. The structure of the posterior

spiracles, peculiar to mature larvae, is seen in Figure 5. Six bundles of greatly convoluted spiracles, each measuring .36 and .13 mm. in average lengths and width, were obliquely arranged in their individual spiracular plate. Each kidney-shaped plate contained two oblique slits which sinuously separated the spiracles. These plates were .65 mm. long and .43 mm. wide. Total length and width of the posterior spiracles was .94 and .65 mm. accordingly. Buttons were recognizable in the spiracles. Cephalopharyngeal skeletons averaged 1.88 and .68 mm. in length and width (Figure 24). Cuterebra fontinella was the proposed species for these larvae. Verification of the Cuterebra species was carried out from taxonomic features found on a puparium which had given rise to an C. fontinella fly.

Puparium. As was previously mentioned the necessity of obtaining the puparial cases related to identified adult flies, was pertinent for confirmation of mature larvae. This confirmation process was possible through studies on the pupal cases which are hardened mature third stage larvae (Figure 25). Thus, any features discovered on pupal cases are transmittible to any mature larvae of that species. The determination of the species Cuterebra fontinella Clark, proposed for the white-footed mouse, has been verified through similar characteristics on the known pupal case.

Fig. 24. The left side of a cephalopharyngeal apparatus of a C. fontinella third stage larva obtained from a white-footed mouse.

Fig. 24

Fig. 25. A dorsal view of a pupal case of C. fontinella after the emergence of a fly. The object above is the pupal cap which has two everted anterior spiracles at its lower border. The puparium is lined internally with a thin white silken membrane. Remnants of the cephalopharyngeal apparatus are seen at the top resting on the venter inside the case.

Fig. 25

Three pupal cases of Cuterebra fontinella, averaging 19.6 mm. in length and 10.6 mm. in width, were obtained from the pupation experiments carried out in the laboratory. Emergent adult flies were sent to Dr. Sabrosky and identification to the above species was confirmed by him. Upon investigation of the pupae, it was found that the pattern and shape of the spines were identical to those proposed for the third instar. Also similar were the structures and measurements of the posterior spiracles and of the oral skeletons. The only difference existing between the third instars and the puparia was the length. It was noted that the pupae had reduced their length, on the average, by 2 mm. because of the process of shrinking and dehydration necessary for pupation. By retrogressing it was possible to survey all the larval stages and see whether the original species determination was valid or not. In the case of Cuterebra fontinella it definitely was.

7. Cuterebra in Sylvilagus floridanus mallurus

a. C. buccata

Third stage. Nine larvae were obtained from Pennsylvania cottontails of which six larvae were recorded as located in the scrota of the hosts. The remaining three larval examples were from the neck. None of the larvae were mature. The average length and width of the larvae were 20 and 11. mm. respectively. After surveying the posterior spiracles of

the larvae, their structures resembled those of C. buccata. Therefore, the nine larvae were suspected of belonging to this Cuterebra species. Studies on the larval segments revealed spines having single and two points. Comparatively however, the number of two pointed spines was not as great as the Cuterebra horripilum. More two-pointed spines were found on the dorsal aspect of the larvae than on the ventral. Larger spines had ranges in length and width of .38 and .50 mm. and .32 to .42 mm. The number of rows of spines taken in segment 7 varied from 15 to 20. Oral mouthparts peculiar to these larvae were quite similar in pattern and approximate measurements to those determined as C. buccata in S. floridanus mearnsii cottontails. Measurements on these cephalopharyngeal skeletons were 2.2 mm. long and .98 mm. wide. Posterior spiracles revealed lengths and widths of 1.08 and 1.44 mm. Individual spiracular plates measured .98 mm. long and .48 mm. wide. Thus, with these similarities, the nine larvae were listed as Cuterebra buccata Fabricius.

Puparium. One pupal case, measuring 21 and 13 mm. in length and width was obtained by pupation experiments. An adult fly identified to this species had emerged from the puparium. Retrogressing it was discovered that the original species presentations peculiar to third stage larvae found in cats, dogs, and rabbits were similar to pupal features.

Three pupal cases, averaging 22.5 mm. long and 11.6 mm. wide, from which adult C. buccata had emerged were submitted

this laboratory by Dr. Sabrosky for study. The previously determined characteristics presented for the mature larvae were confirmed upon examination of the puparia.

8. Cuterebra in Sylvilagus floridanus mearnsii

a. C. horripilum

First stage. A single example of this instar was obtained during the Mason game farm study. The length and width of the early larva were 5 and 3 mm. respectively. Single and multipointed spines were seen on the larval segments. However, the former spines were more predominant. These spines ranged from .12 to .17 mm. in length and .043 to .086 mm. in width using the larger spines as examples. Pattern of the posterior spiracle was similar to those in Figure 7 which is peculiar to early instars in Felis domestica. The length and width of the posterior spiracles were .50 and .36 mm. respectively. Individual spiracles measured .19 and .09 mm. for the length and width. The arrangement and number of spines were similar to those of larvae found in the cat. Oral mouthparts revealed a morphology similar, if not identical, to that in Figure 9. The oral skeleton measured .68 and .36 mm. in length and width. This example was relegated to Cuterebra horripilum.

b. C. buccata

Second stage. Two sources of larvae from cottontails were obtained in this stage; one from New Jersey rabbits and the other from Michigan cottontails. The color of the larvae was white and they had an average length and width of 14.0 and 5.0 mm. respectively. Larvae from New Jersey had long delicate single pointed spines measuring .35 and .21 mm. for the average length and width. Further studies on the spines revealed a pattern and number similar to that found in the cat. However, the posterior spiracles resembled that of the dog Cuterebra in size and pattern (Figure 17). The spiracles were smaller than those of C. horripilum, having average length and width of .42 and .29 mm. They were negative for buttons. Also the oral skeleton was discovered to be smaller than C. horripilum in length and width, measuring .75 and .36 mm. The oral mouthparts of the New Jersey cottontail larvae resembled that of the Canis species (Figure 16). Thus, with this comparative presentation it seemed that the larvae occurring in New Jersey rabbits were Cuterebra buccata.

Second stage larvae collected from Mason rabbits appeared similar to those of New Jersey cottontails and to those larvae from Canis familiaris. These similarities are seen in the shape, measurement and arrangement of spines. Also the oral skeleton and posterior spiracles were respectively identical

in structure and measurements to those of the cat, dog and New Jersey rabbits. Therefore, these larval stages peculiar to Michigan cottontails were reported as C. buccata.

a. C. horripilum

Third stage. Twenty-five larvae collected from S. floridanus mearnsii cottontails were determined as C. horripilum. The greatest length and width was a mature larvae with 32 and 17 mm. respectively. It was noted upon investigations that one and two pointed spines existed on the larvae. They revealed posterior spiracles and oral skeletons similar to those of the previously discussed C. horripilum in cats. Measurements on these structures were larger, in average, than those recorded in cats. Posterior spiracles were 1.5 mm. long and .84 mm. wide. The length and width of the posterior spiracular plates were 1.0 and .46 mm. accordingly. Oral skeletons revealed an average length and width of 2.6 and .92 mm. Larger spines found on these larvae gave lengths ranging from .38 to .50 mm. and widths ranging from .28 to .32 mm. These measurements were greater than those peculiar to third instars in cats which indicates that these larvae were more mature. However, the collection of C. horripilum third instars were discovered to be in their early and mid-phases of that stage. No mature third stage larvae were studied in this species.

b. C. buccata

Third stage. Twenty-two larvae had predominantly single pointed spines on their segments. Approximately one dozen of these larvae were in the mature state of the third stage. Ranges for their length and widths were 25 to 30 mm. and 14 to 18 mm. Larger spines revealed ranges of .38 to .45 mm. in length and .28 to .32 mm. in width. The posterior spiracles had lengths and widths averaging 1.08 and 1.44 mm. Spiracular plates were 1.00 mm. long and .58 mm. wide. Oral mouthparts revealed an average length and width of 2.5 and .98 mm. respectively. The previous measurements were taken from mature larvae which were determined as C. buccata and resembled those discussed in cats.

a. C. horripilum

Puparium. Five pupal cases, averaging 26 and 17 mm. in length and width, were obtained from pupation experiments. The adult flies which emerged from the puparia were confirmed by Dr. Sabrosky as C. horripilum species. Studies on the pupae revealed species characteristics identical to those presented for the third instars occurring in cats and cottontails. Segment 11 in the posterior rows of spines had intensely orange colored spines.

IV. DISCUSSION

There appears to be no evidence in the literature of intensive studies on Cuterebra larvae occurring in cottontails, especially in Michigan rabbits. In general, pertinent references have been minor observations encountered accidentally in zoological studies, such as Wildlife and Mammalogy. Though these observations are beneficial, they are merely minute fragments of the Cuterebra picture and may be subjected to various interpretations by different zoological workers. It is thought that this two-year cuterebrid larval investigation of Sylvilagus floridanus mearnsii is, at present, the most specific in relation to life history, ecology and systematics.

A. LIFE HISTORY

The mode of infection hypothesis that the cuterebrid eggs are laid on food plants of the host, and when the animal feeds, the eggs are ingested (Townsend, 1935) was in serious doubt because the acquisition of a cuterebrid infection in a caged cottontail not exposed to natural vegetation. The probability that the sampling data of Cuterebra were specifically limited in these mode of infection experiments, involving 22 bait pen rabbits, was realized. However, with

the obtained data acquired by these studies, it is believed that the mode of infection does not entail the eating of cuterebrid egg-infested vegetation by the host. The hypothesis presented by Allison (1953) that rabbits acquire infections by brushing against vegetation having first stage larvae is also deemed invalid on the basis of the above experimental results.

As a result of the life history study, it is not maintained that the adult females lay their eggs on definitely predisposed areas of the hosts such as the neck or scrota. Nor subsequently, that these eggs hatch into first instars which dig through the skin, using their oral hooks, and directly settle subcutaneously in these areas as final infection sites. Evidences supporting this belief were that no first stage larval swellings had scars overlying the tumorous areas (indicating larval entry) and that no early instars had air holes which the larvae would use seemingly to facilitate breathing by the posterior spiracles. Beamer (1950) stated that the eggs may be seen with the naked eye. In this study no eggs were observed on these predisposed areas in 170 Mason Farm cottontails, especially in those rabbits recaptured within 15 days time between an obvious negative condition and an obvious third stage larval infection. Dalmat (1943) observed that as many as 450 to 1000 eggs have been counted from female cuterebrid flies. It would seem that oviposition on predisposed areas of the host by adult females is unlikely due to

the few larval infections found in these areas of the host. These few infections would infer that the cuterebrid eggs were quite vulnerable to environmental conditions. To the contrary, Parker and Wells (1919) discovered that the eggs lived six months in a laboratory container and even then some were viable.

Suggestion of cuterebrid larval migration was found in the study on the duration of instars. Two third instars appeared in one side of the neck of a cottontail, diagnosed as negative in that area a week previously. With the possibility of migration in cottontails, it was thought that evidences of migration could be found upon autopsy in infected dead rabbits. It was known that the larvae of Hypoderma lineatum showed migratory routes in the tissues of their hosts and in this manner larval migrations were anatomically traced. In sixty-two cottontails autopsied no evidences of migration such as routes or larval stages were ever seen in muscles, mesenteries or other body areas. However, inasmuch as early instars were found subcutaneously in the neck, it was believed that the migration to their final infection sites was done by the first instar. First stage instars were found in this study molting to the second stage and the second molting to the third stage all in one infection site. In one case an early instar was extracted from a caseous cyst which was discovered underlying the skin. Externally there was no scar. Thus, it was maintained that the larva was inactivated by

the reactions of the host when the parasite reached its final location through internal means in the host. Another finding that suggested migration was that larvae in a multiple infection of a rabbit were discovered not to be in the same phase of larval development. Larvae in the same area of the host were observed upon maturation to drop during a period of from one to three days. This condition, it is believed, would not be prevalent if eggs were laid by flies in predisposed areas. Rabbits with many larvae were held in the laboratory after the dropping period to see if other larvae would appear which might indicate more than one infection in the host. No detained rabbits showed this type of infection.

Because of the shortness of the life cycle, approximately one month, ascertained by Scott and Snead (1942), it was felt that the migration was a short one. Reason for this belief was that since the time of the third instar in the host was determined in this study to be 15 days, few days would be left in a month to allow for an extensive migration for the first stage and molting to the second and consequently to the third stage. Possible areas of short migrations in relation to final infection sites were examined. Oral and nasal passages were checked in twenty-five rabbits along with esophageal and tracheal areas. Also the soft abdominal skin adjacent to the scrota of male cottontails were investigated. Probably due to the sampling, negative results were obtained.

It was discovered in this study that some cottontails had larval infections in the areas of the cheek. Records of anatomical locations of infections in the veterinary samples submitted to the laboratory were from the eyelid, nares, cheeks, chin and neck of domestic mammals. Philip and associates (1955) found multiple larval infections occurring in Nevada jack rabbits (Figure 1) in the region of the eyes, chin and cheeks. It seemed likely from the above examples of larval infection sites that oral and nasal cavities are the sites of primary infection initiating a short migration of first stage larvae to these infection sites. This contention is partially supported by the fact that no evidences of larval migration were seen in the areas other than the head and the neck.

The use of air hole diameters in determining the age of the larvae found in infected cottontails is believed to be of practical significance. It was discovered and applied in this study for the first time and was shown to be useful in inspecting infected cottontails for laboratory studies on duration of instars. As the larval air hole approached that diameter peculiar to mature larvae, the host was carefully watched for the dropping of the mature larvae and their pupation in the soil trays to record their behavior and time of pupation.

B. ECOLOGY

There is no apparent record in the literature on the seasonal periodicities of immature cuterebrid larval stages in Michigan cottontails. It was found in this study that the first appearance of a first instar in a Michigan cottontail was on June 28, 1953. A second instar in a wild rabbit was recorded on July 25, 1953. Because of the paucity of earlier instar larvae, it is felt that these months do not respectively show the seasonal periodicities of the mentioned larval stages. In this study it has been determined that the seasonal peaks of mature larvae were found between August 1 and 20 (Graphs 2, 4, 5, 6, 7, 8) and that the length of the Cuterebra life cycle in the host was approximately one month. Thus, it is believed that the seasonal periodicity of the first instar larvae should occur about one month previous to the seasonal peaks of mature larvae in the early part of July.

From this study it was believed that certain conditions conducive to cuterebrid infections existed. The best situation for cuterebrid infections was thought to be the nest or burrow of the host. This belief was partially supported by the fact that more larval infections occurred in areas having the highest density of rabbit burrows. Young cottontails determined by weight measurements (Petrides, 1951) to have recently left their nests were discovered infected with

Cuterebra larvae. Also adult female rabbits which were in the process of lactating were found with infections.

Periods of aestivation by cottontails were noted prior to cyclic peaks of larval infections. Rabbits at these times were seen in their burrows or shady areas during the day hiding from the hot sun rays of July. Perhaps it was due to the subsequent increase in rabbit captures that these high numbers of cuterebrid infections occurred. Nevertheless, the fact that these "highs" did exist after these aestivations revealed a possible relation between adult flies and infections in burrows or shady areas. The capture of an adult fly atop a rabbit burrow may or may not add further evidence to this.

In relation to the high larval incidence of Cuterebra in areas having the highest density of burrows, a fact noted afterwards was that the soil type of these areas was of a sandy nature. The best example was sandy loam. Results obtained on the influence of soil in pupation experiments revealed that mature larvae burrowed best and fastest in sandy soils. It was concluded therefrom that the reason for greater larval infections in areas of greater number of burrows was that more successful pupations occurred and more cuterebrid flies emerged in the sandy areas. This ecological situation is believed to be contributory to more infections of the rabbits in their burrows.

It was felt after the first trapping program of 1953 that the sampling data of Cuterebra larvae were rather limited in number. This was believed due, in part, to the inexperience of trapping cottontails on the Mason farm and of interpreting cuterebrid material. However, comparing results obtained from the second trapping program at Mason in 1954 and the cottontail studies on the Kellogg Station, it was discovered that the number of cuterebrid infections in cottontails was normally small. Added to this fact was the low natural population of cottontails encountered during the summer trapping which decreased the cuterebrid sample material further. Though seemingly this was a bad time of the year to study this host-parasite relationship, it was the only valid time to do so ecologically. Graphs 2, 4, 5, 6, 7, 8 on the seasonal incidences of larval infections revealed this ecological situation to be true.

It was thought at the onset of this study that adult cuterebrids could be lured into fly traps with the use of various baits. Curran (1934) stated that the adult cuterebrids have poorly developed mouth parts and that the adult flies were believed incapable of feeding. Thus, it would appear that the Cuterebra flies were only reproductive in nature. However, inasmuch as there was no previous work done on baiting cuterebrid flies, the experiment was established to observe whether the adult flies would employ baits similar

to those used for Diptera, such as horse flies. The results obtained in this bait experiment were negative.

However, applying the ecological knowledge of the adult flies that they frequent the host's burrows (Townsend, 1915), an adult fly was captured in an unbaited fly trap atop a cottontail burrow. Thus, it appeared from this study that the baiting of fly-traps for capture of adult cuterebrids was not the proper method. By employing ecological situations pertinent to the fly, results were obtained. The possibility that this capture was merely a chance condition was realized. However, the chances seemed to be increased by placing the trap in that ecological site.

C. SYSTEMATICS

Another subject presented for discussion is the presence or absence of the buttons in the posterior spiracles of cuterebrid larvae. The status of the buttons is necessary in order to classify the Cuterebra posterior spiracles as to schizotreme or ptychotreme types of stigmata for dipterous larvae presented by MacGregor (1914). Greene (1925) stated that no buttons were found in the Cuterebridae. The stage of the larval example described by that author was not specifically given and conclusions therefrom were not obtained. Knipling and Brody (1940) and Dalmat (1942) have shown buttons in third instars. From the systematic study of various

instar examples of Cuterebra stigmata, it was found that no distinct buttons as shown by the three above workers were seen in the early larval instars. In this study, buttons were observed in the third instar stigmata of C. horripilum, buccata, fontinella and the Cuterebra species of Nevada jackrabbits. MacGregor (1914) in his definition of a ptychotreme stigma related that the button was always present and usually very distinct. The above author also stated that the ptychotreme type had a very marked ring which is the thickened periphery of the stigmal plate. In the present study no heavy ring was seen peculiar to this type of stigma. However, one distinct feature pertinent to ptychotreme stigma was observed. This morphological characteristic was that of a convoluted chain, either broken into segments or unbroken, which was situated within the ring. It appeared that the cuterebrid stigma or posterior spiracle did not distinctly classify into either type as both types were reflected in the Cuterebra. It is believed that an intermediate evolutionary position exists for the cuterebrid stigma in between the schizotreme and the ptychotreme types of dipteran stigmata approaching the ptychotreme stigmata. Evidence of an evolutionary link was seen in the earlier stigma of second stage larvae of C. fontinella which had straight rods of spiracles peculiar to the schizotreme example (Figure 6). It is hoped that a more complete study on this taxonomic subject will be carried out in the future.

Having a sizeable sample of cuterebrid larvae (127 representatives), it was possible to determine new ranges and larger coverage of morphological characteristics to present for larval species determinations. It was with this idea in mind that these results were compared, with those of previous workers (Tables XIV, XV, XVI). Knipling and Brody (1940) in their presentation of C. buccata and C. cuniculi= horripilum offered procedures and measurements which were used in this study. The present work contributed to the taxonomy of the first and second stages of C. horripilum in which new measurements on morphological structures were obtained. The above authors presented in their taxonomic studies the greatest lengths and widths of the cephalopharyngeal skeletons of C. horripilum and C. buccata as 5.5 mm. and 2.7 mm. and 4 mm. and 1.8 mm. respectively. Compared to the measurements obtained in this study (Table XVI) they appear to be twice as great. Oral skeletons here were measured by recording the dimensions on the heavily chitinized structures, that is the oral, hypostomal sclerites and the dorsal and ventral arches. The dorsal and ventral cornua were not considered in the measurements, as it was believed that these softer structures would be damaged upon dissection by inexperienced workers and would result in abnormal dimensions. It was discovered that if the dorsal and ventral arches were measured, the measurements would approximately equal those

TABLE

A COMPARISON OF FIRST INSTAR CU
WITH MEASUREMENTS OBTAIN

C. horripilum		C. bu
Boisvenue (Average of 6)	Knipling & Brody (1940)	Boisvenue
Aver. length 4.88 mm. " width 2.38 mm.	No First Instars	No First Instars
Spines: 1 and 2 pointed spines Spine length .12-.17 mm. " width .04-.08 mm.		
Posterior Spiracles: Length .48-.50 mm. Width .36-.38 mm.		
Oral Skeletons: Length .68-.72 mm. Width .32-.36 mm.		

XIV

TEREBRA SPECIES CHARACTERISTICS
ED BY TAXONOMIC WORKERS

ccata	C. fontinella	C. peromysci	Cuterebra sp.
Knipling & Brody (1940) No First Instars	Boisvenue Moulting First Instar 1 larva only in which only oral skeleton was obtained	Dalmat (1942) 1 Larva Length 4.2 mm. Width 3.2 mm.	From Nevada Jack Rabbits No First Instars
	Oral Skeleton: Length .48 mm. Width .17 mm.	No record of oral skeleton	

TABLE

A COMPARISON OF SECOND INSTAR CU
WITH MEASUREMENTS OBTAIN

C. horripilum		C. bu
Boisvenue (average of 12 larvae) L. 11.0 mm. W. 4.5 mm. Full grown larvae: L. 15 mm. W. 6 mm.	Knipling & Brody (1940) No Second Instars	Boisvenue (average of 3) L. 14 mm. W. 5 mm.
Spines: 1 and 2 pointed L. .35 mm. W. .16 mm.		Spines: 1 point L. .33 mm. W. .19 mm.
Posterior spiracles: L. .65 mm. W. .42 mm.		Posterior spiracles: L. .45 mm. W. .31 mm.
Oral skeleton: L. .86 mm. W. .43 mm.		Oral skeleton: L. .74 mm. W. .36 mm.

XV

TEREBRA SPECIES CHARACTERISTICS
ED BY TAXONOMIC WORKERS

ccata	C. fontinella	C. peromysci	Cuterebra sp.
Knipling & Brody (1940) (average of 2) L. 10.5 mm. W. 4.5 mm.	Boisvenue (average of 3) L. 9.5 mm. W. 4.3 mm.	Dalmat (1942) (average of 4) L. 9.5 mm. W. 3.0 mm.	Nevada Jack rabbits Boisvenue 1 larva L. 14 mm. W. 6 mm.
Spines: 1 point No record	Spines: 1 and multi-pointed L. .09 mm. W. .03 mm.	No spines recorded	Spines: 1 and multi-pointed L. .29 mm. W. .17 mm.
No record	Spiracular plate: L. .14 mm. W. .17 mm.	Spiracular plate: L. .12 mm. W. .18 mm.	Posterior spiracles: L. .26 mm. W. .15 mm.
L. 1.6 mm. W. .75 mm.	Oral skeleton: L. 1.22 mm. W. .65 mm.	Oral skeleton: L. 1.1 mm. No width recorded	Oral skeleton: L. .72 mm. W. .29 mm.

TABLE

A COMPARISON OF THIRD INSTAR CU
WITH MEASUREMENTS OBTAIN

C. horripilum		C. bu
Boisvenue (average of 33) L. 32 mm. W. 17 mm.	Knipling & Brody (1940) (Number?) L. 35-43 mm.	Boisvenue (average of 37) L. 15-30 mm. W. 8-18 mm.
Spines: 1 and 2 pointed L. .38-.57 mm. W. .28-.48 mm.	Spines: L. .65-80 mm. W. .55-65 mm.	Spines: 1 point (rare 2) L. .38-.45 mm. W. .28-.32 mm.
Posterior spiracles: L. 1.6 mm. W. .84 mm.	L. 1.1 mm. W. 0.5 mm.	Spiracular plates: L. 1.00 mm. W. .58 mm.
Oral skeleton: L. 2.2-2.6 mm. W. .92-1.08 mm.	Greatest L. 5.5 mm. W. 2.7 mm.	Oral skeleton: L. 2.0-2.5 mm. W. .75-.98 mm.

XVI

TEREBRA SPECIES CHARACTERISTICS
ED BY TAXONOMIC WORKERS

ccata	C. fontinella	C. peromysci	Cuterebra sp.
Knipling & Brody (1940) (average of 4) L. 26-32 mm. W. one-half	Boisvenue (average of 5) L. 14.6 mm. W. 7.9 mm.	Dalmat (1942) (average of 16) L. 20-25 mm. W. 7.5-7.8 mm.	Jack-rabbits of Nevada Boisvenue (average of 18) L. 25.4 mm. W. 11.2 mm.
Spines: 1 point (2) L. .40-.45 mm. W. .30-.35 mm.	Spines: 1-6 points L. .28-.32 mm. W. .19-.21 mm.	Spines: 1-4 points L. .36-.41 mm. W. .25-.30 mm.	Spines: 1-3 points L. .38-.58 mm. W. .32-.54 mm.
L. .88 mm. W. .45 mm.	Spiracular plates: L. .65 mm. W. .43 mm.	Spiracular plates: L. .91 mm. W. .33 mm.	Posterior spiracles: L. 1.01-1.20 mm. W. .65-.80 mm.
Greatest L. 4 mm. W. 1.8 mm.	Oral skeleton: L. 1.88 mm. W. .68 mm.	Oral skeleton: L. 2.5 mm. No width recorded	Oral skeleton: L. 2.30-2.44 mm. W. 1.00-1.08 mm.

presented by Knipling and Brody (1940). Thus, it was maintained that the most stable procedure for recording measurements on the oral skeletons was that of considering the heavily sclerotized structures which make up the skeleton.

Knipling and Brody (1940) made studies on the anterior spiracles which were not used in this study. It was felt that since measurements of the structures were only recorded, the anterior spiracles were not significant systematically as posterior spiracles, oral skeletons and spines whose morphology and measurements were determined.

Dalmat (1942) presented first, second and third stage larval descriptions of C. peromysci. The above author stated that the adults of C. peromysci closely resemble those of C. fontinella. It was found in the larval study of these two species presented in Tables XIV, XV, XVI, that the larvae also closely resembled one another. However, differences were seen in the second instar which Dalmat (loc. cit.) maintained had a transverse band of spines on anterior margins of all but the first segment of C. peromysci. Present observations on C. fontinella showed no transverse band of spines on the anterior margins of segments 10 and 11. The spines found on the larval segments of the third instar of C. fontinella had 1 to 6 points, whereas those recorded for C. peromysci by Dalmat (loc. cit.) were 1 to 4 points. Also it was discovered that the mature larvae of C. fontinella had a

reddish appearance due to the spines being colored red. Dalmat (loc. cit.) does not state the color of his mature larvae, but it is assumed that it was a normal dark brown or he would have mentioned it. Measurements recorded on mature larvae of C. fontinella and peromysci (Table XVI) indicated that the C. peromysci were generally larger.

The predominant species of Cuterebra found on Michigan cottontails was C. horripilum. Of the twenty-five larvae collected from the cottontails, 18 were determined to be C. horripilum. The remaining seven were C. buccata.

Twenty-six larval specimens were obtained from Pennsylvania cottontails. This sample contained twenty larvae classified as C. buccata. The remaining larvae were C. horripilum. Thus, on the material collected it would appear that C. buccata is the predominant species occurring in Pennsylvania.

In the white-footed mouse, thirteen larval specimens collected in Michigan were determined as C. fontinella. Thus, it is thought that the predominant cuterebrid species occurring in the Michigan Peromyscus leucopus is C. fontinella.

The most frequent larval species collected from Felis domestica was C. horripilum. Thirty-five larvae out of fifty were relegated to this species. C. buccata were the remaining species.

Referring to the Appendix, Cuterebra fontinella and horripilum have been recorded in Michigan. However, the

incidence of these species in their respective hosts could not be ascertained from the literature. Records from Pennsylvania have shown that C. buccata has been found there. New Jersey cottontails were discovered to have C. buccata larvae, which was confirmed with the finding of that species in that state. However, there are apparently no records of C. horripilum in that state. Thus, it is presented here that in the eleven larvae received from Dr. Hansens, four larvae were identified as C. horripilum.

As a result of the systematic study, it was felt that identification of larvae to species should be carried out through a known adult fly-pupa series. Inasmuch as the taxonomy of adults was more stable than the larvae, the prime standard of verification was the fly. Thus with a definitely identified adult fly which had emerged from its pupa, it was possible to study pupal morphological characteristics which similarly reflected the situation in the mature third instars. With features of the third instars it was not hard to work back to the second and first stages, assimilating the characteristics originally determined from the puparium. Through comparison of these features among various larvae, a key was constructed (page 141) which distinctly separated three cuterebrid species, namely C. fontinella, C. buccata and C. horripilum. The unidentified cuterebrid species found on Nevada jack-rabbits is also presented in the key. In employing this

adult fly-pupa series method of identifying the larvae of Cuterebra to species, it is rather difficult to deny the validity of the species found in this manner.

A Key to the Species of Cuterebra Larvae in This Study

1. Body white to cream-colored; spines arranged in several somewhat irregular rows that encircle the body; posterior spiracular plates bisinuate; larval length 2-15 mm., width 1-6 mm. ----Cuterebra spp. (First and Second Stage) 2

   Body light brown to black; spines or pointed scales closely set covering all of the surface of each segment; posterior spiracular plates divided into three divisions; larval length 15-43 mm., width 8-18 mm.----------
   ----------Cuterebra spp. (Third Stage) 6

2. Larval length 2-5 mm., width 1-2 mm.; spines showing a haphazard arrangement in first four anterior segments; 1 row of anterior spines on segments 10 and 11, no intermediate spines; spiracular plates (Fig. 26), .48 mm. long and .36 mm. wide; cephalopharyngeal apparatus (Fig. 27) .72 and .32 mm. long and wide (cat, cottontail)----
   ----------C. horripilum (First Stage).

   Larval length 6-15 mm. width 3-6 mm.; spines not showing a haphazard arrangement in first four anterior segments
   ----------Cuterebra spp. (Second Stage) 2a

2a. Transverse row of spines on all but first segment-- 3

   Transverse row of spines lacking on segments 1, 10, 11
   ---------- 5

Fig. 26. Posterior spiracular plates of a first instar larva of C. horripilum (reduction of Figure 8).

Fig. 27. The left side of the cephalopharyngeal apparatus dissected from a first instar larva of C. horripilum (reduction of Figure 9).

Fig. 28. Posterior spiracular plates of a second instar larva of C. buccata (reduction of Figure 17).

Fig. 29. The left side of the cephalopharyngeal apparatus dissected from a second instar larva of C. buccata (reduction of Figure 18).

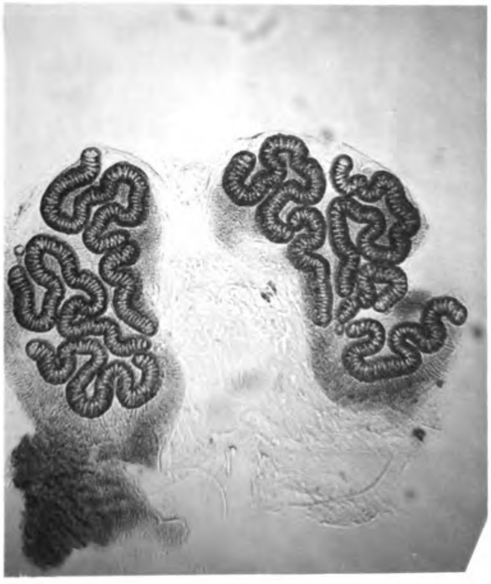


Fig. 26

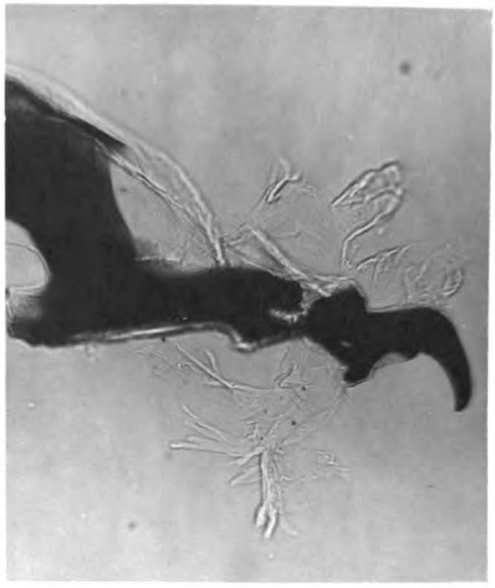


Fig. 27

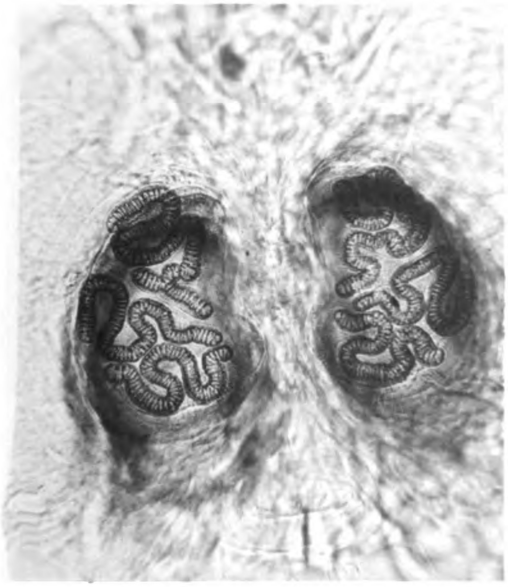


Fig. 28

Fig. 29

3. All spines single pointed on larval segments; spiracular plates .12 mm. long and .18 mm. wide; cephalopharyngeal apparatus length 1.1 mm. (rodents, Dalmat, 1942)-------
----------C. peromysci
Spines predominantly single pointed with infrequent two pointed spines on larval segments (larvae not on rodents)
---------- 4

4. Single pointed spines greatly predominate, only rarely two pointed spines on larval segments; posterior spiracles (Fig. 28) .43 mm. long and .32 mm. wide; cephalopharyngeal apparatus (Fig. 29) .72 mm. long and .36 mm. wide (dog, cat, cottontail)----------C. buccata
Single spines predominate with two pointed spines more frequent; posterior spiracles (Fig. 30) .65 mm. long and .42 mm. wide; cephalopharyngeal apparatus (Fig. 31) .86 mm. long and .43 mm. wide (dog, cat, cottontail)-------
----------C. horripilum

5. Three pointed spines infrequent; spiracular plates (Fig. 32) .13 mm. long and .09 mm. wide; oral skeleton (Fig. 33) .72 mm. long and .29 mm. wide; larvae found on jackrabbits of Nevada----------Cuterebra sp. (unidentified)
Three pointed spines frequent; spiracular plates (Fig. 34) .14 mm. long and .17 mm. wide; cephalopharyngeal apparatus 1.22 mm. long and .65 mm. wide (rodents)-----
----------C. fontinella

Fig. 30. Posterior spiracular plates of the second instar larva of C. horripilum (reduction of Figure 11).

Fig. 31. The right side of the cephalopharyngeal apparatus of the second instar larva of C. horripilum (reduction of Figure 10).

Fig. 32. Posterior spiracular plates of the second instar larva of the Nevada jack-rabbit Cuterebra species (reduction of Figure 18).

Fig. 33. The right side of the cephalopharyngeal apparatus of the second instar larva obtained from Nevada jack-rabbits (reduction of Figure 19).

Fig. 34. Posterior spiracular plates of the second instar larva of C. fontinella (reduction of Figure 6).

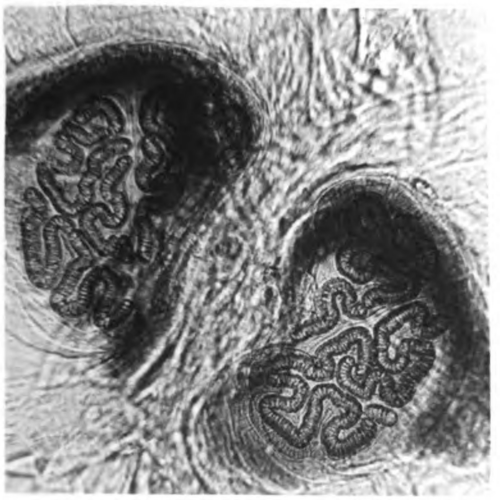


Fig. 30

Fig. 31

Fig. 34

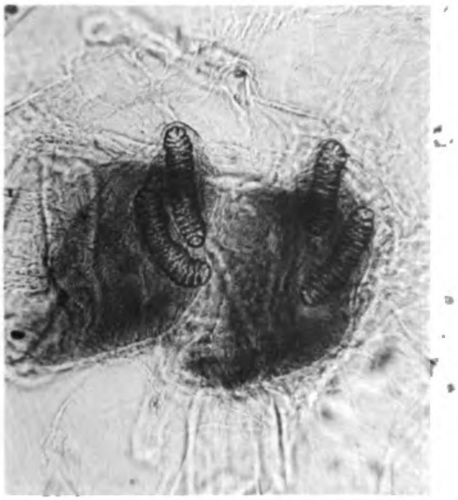


Fig. 32

Fig. 33

6. Posterior spiracular plates not distinctly separated into 3 divisions (Fig. 35); posterior spiracles 1.6 mm. long and .84 mm. wide; cephalopharyngeal apparatus (Fig. 36) 2.4 mm. long and 1.0 mm. wide; single and two-pointed spines on larval segments; orange colored spines on posterior margin of segment 11 (cat, cottontail)----------C. horripilum
Posterior spiracular plates distinctly separated into 3 divisions---------- 7

7. Spines predominantly single pointed, very rarely two pointed on larval segments, .38 - .45 mm. long and .28 - .32 mm. wide; lacking orange colored spines on segment 11; posterior spiracular plates (Fig. 37) 1.00 mm. long and .58 mm. wide; cephalopharyngeal apparatus (Fig. 38) 2.2 mm. long and .87 mm. wide (cat, cottontail)----------C. buccata
Spines commonly multipointed on larval segments---- 8

8. Larvae from jack-rabbits of Nevada; spines having one to three points; posterior spiracles with "macaroni" pattern (Fig. 39) 1.10 mm. long and .74 mm. wide; cephalopharyngeal apparatus (Fig. 40) 2.37 mm. long and 1.04 mm. wide----------Cuterebra sp. (unidentified)
Larvae from rodents----------9

9. Spines having one to four points, .36 - .41 mm. long and .25 - .30 mm. wide; posterior spiracular plates .91 mm. long and .33 mm. wide; cephalopharyngeal apparatus 2.5

Fig. 35. Posterior spiracular plates of a mature third instar of C. horripilum (reduction of Figure 12).

Fig. 36. The right side of the cephalopharyngeal apparatus from a mature third instar of C. horripilum (reduction of Figure 13).

Fig. 37. Posterior spiracular plates of a mature third instar of C. buccata (reduction of Figure 14).

Fig. 38. The left side of the cephalopharyngeal apparatus from a mature third instar of C. buccata (reduction of Figure 15).

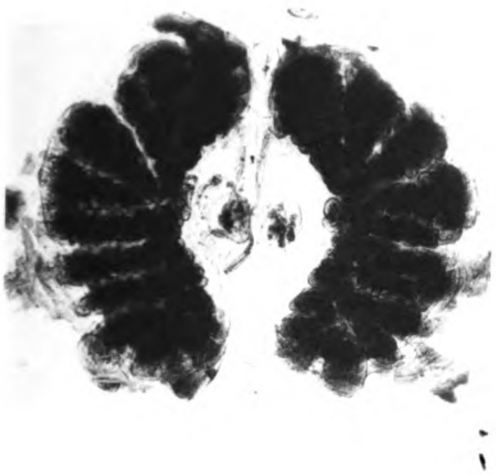


Fig. 35

Fig. 36

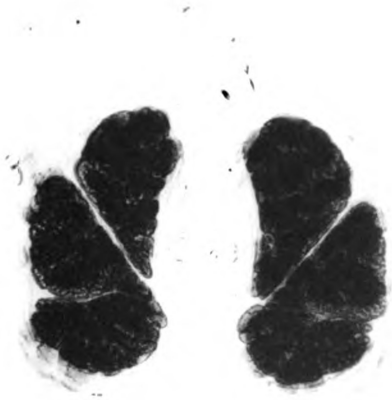


Fig. 37

Fig. 38

Fig. 39. Posterior spiracular plates of a third stage larva from the Nevada jack-rabbit (reduction of Figure 20).

Fig. 40. The left side of the cephalopharyngeal apparatus of a third stage larva from the Nevada jack-rabbit (reduction of Figure 21).

Fig. 41. Posterior spiracular plates of a mature third stage larva of C. fontinella (reduction of Figure 5).

Fig. 42. The left side of the cephalopharyngeal apparatus from a mature third stage larva of C. fontinella (reduction of Figure 24).

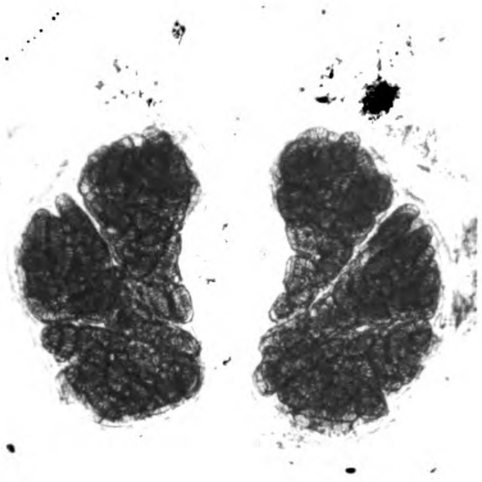


Fig. 39

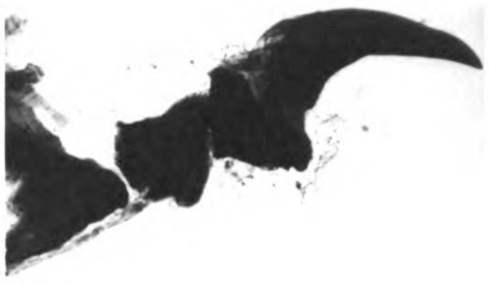


Fig. 40

Fig. 41

Fig. 42

long, no width recorded (white-footed mouse; Dalmat, 1942)----------C. peromysci

Spines having one to six points, .28 - .32 mm. long and .19 - .21 mm. wide; spines red giving larvae a reddish appearance; posterior spiracular plates (Fig. 41) .65 mm. long and .43 mm. wide; cephalopharyngeal apparatus (Fig. 42) 1.88 mm. long and .68 mm. wide (white-footed mouse)----------C. fontinella

SUMMARY

The life history and ecology of Cuterebra species, occurring in Michigan cottontails, were studied in part by means of a two-year live trapping program of rabbits. Field observations on adult cuterebrid flies and rabbits were also carried out. One hundred and seventy rabbits were captured and were screened for Cuterebra larval infections. Infected cottontails were brought into the laboratory and studied for phases of the life cycle.

Records on the duration of third stage larvae in the cottontails related an average of 15 days. Concurrent with the above study, it was found that the air hole diameters of larvae reflected the phases of the third stage larvae while in the host.

Mode of infection studies were made in the field using twenty-two penned cottontail and domestic rabbits in areas having high larval incidences. A positive larval infection was obtained in a penned cottontail which was not exposed to natural vegetation.

The primary site of Cuterebra larval infections in 187 southern Michigan infected cottontails was in the neck. Other anatomical areas recorded were the scrota, cheeks, back and shoulders.

Suggestion of larval migration was observed when two third stage larvae appeared on the right side of the neck of an isolated cottontail which was externally determined negative in that area a week previously. A large tumor appeared first in the area followed by two larval air holes overlying the tumor. No evidence of extensive larval migration was found in sixty-two cottontails which were autopsied throughout the Cuterebra cycle.

The time recorded for the obvious appearance of third stage larvae in cottontails from a negative determination was 22 days.

First, second and third stage larvae were discovered in the cottontails which gave seasonal periodicities in their hosts. The seasonal periodicity of third stage larvae was determined to be between August 1 to 20. First stage larvae were believed to have a seasonal periodicity a month previous to the third stage larvae in the early part of July.

Seasonal incidence of cuterebrid larvae in southern Michigan juvenile cottontails was determined to be between August 1 and 20. For adults, it was found to be between July 20 to 30. In general, the juvenile cottontails had a higher incidence of Cuterebra larvae than adult rabbits. Also the number of warbles per infected rabbit was found to be the greatest when there was a peak in the incidence of Cuterebra larvae among the cottontails.

Field notes on pupation showed that mature larvae which had recently dropped from their hosts were able to survive 9 to 13 days before pupating. Depths recorded on 27 burrowing larvae offered a range of 1 1/2 to 2 1/2 inches in sandy soils. Field pupation experiments revealed that no two-year cycle existed for the pupae. Also one generation a year was shown to exist in Michigan for Cuterebra, regardless of exposing the pupae to artificial or natural conditions prior to the emergence of the flies. Longevity of adult flies were observed in laboratory pupation jars to range from 1 to 5 days.

Higher numbers of Cuterebra infections were recorded in areas having sandy soils. Also these sandy areas showed greater numbers of rabbit burrows. This situation was believed conducive to good pupation and consequently, successful emergence of adult flies with excellent conditions for larval infections in the rabbit burrows.

Vegetative sites such as dense sumac bushes, willow swales and clover fields were associated with adult cuterebrid flies.

One hundred and twenty-seven cuterebrid larvae, collected from cottontails, cats, dogs, jack-rabbits, and white-footed mice, were comparatively studied and taxonomic differences were presented for each instar of individual Cuterebra species studied. The results obtained in the systematic study of larvae were compared with the findings of previous workers.

Buttons, peculiar to posterior spiracles of some dipterous larvae, were seen in the cuterebrid examples investigated.

A key is presented which separates the various instars of the individual larval species determined in the systematic study. Species of Cuterebra determined in this study were buccata, fontinella, and horripilum. An unidentified species, found on Nevada jack-rabbits was described and its larval characteristics are presented for comparison with the known cuterebrid species. No pupa-adult fly series were obtained for the identification of the Nevada species. However, they were obtained for the three identified species.

Verification of the three Cuterebra species was accomplished by means of a puparium from which a known identified adult fly emerged. With the morphological characteristics of the puparium it was possible to retrogress to the third instar and subsequently to the first instar of that species.

Larval species found in Felis domestica were C. buccata and horripilum, with a preponderance of the latter species. Cuterebra buccata was recorded from Canis familiaris. Michigan cottontails had both cuterebrid species, with C. horripilum predominating. The only larval species obtained from the white-footed mouse of Michigan was C. fontinella.

A check-list of the Cuterebra species of the western hemisphere is presented with records of their geographic locations and the scientific names of their hosts.

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APPENDIX

A Check-list of Cuterebra With Their Hosts and Geographic Locations

Cuterebra albifrons Swenk (= to C. princeps, Townsend, 1917).

Lepus californicus and Sylvilagus floridanus; Arizona Calif., Nevada, Wyo., New Mexico; Townsend, 1917.

C. abdominalis Swenk (= to C. horripilum, Townsend, 1917).

C. aldrichi Austen.

Neotoma fuscipes f.; California; Austen, 1933.

C. almeidai.

Distribution in Brazil; Guimaraes, 1941.

C. americana Townsend.

Lepus alleni a.; Arizona; Vorhies and Taylor, 1933.

Lepus californicus c.; Arizona; ibid.

Lepus palustris; Mexico; Brauer, 1863.

Distribution also in New Mexico; Skinner, 1903.

In California; Ferris, 1920.

C. analis Macquart (= to C. emasculator, Van Der Wulp, 1888).

Distribution in Mexico; Swenk, 1905. In Brazil, Macquart, 1843. In Arizona; Austen, 1895.

C. apicalis (= to C. analis, Guérin-Méneville, 1829).

Distribution in North America and South America; Guérin-Méneville, 1829.

C. approximata Wlk.

Distribution in Arizona; Townsend, 1917. In Mexico and Guatemala; Van Der Wulp, 1888. In Vancouver, B. C.; Austen, 1895.

C. atrox Clk. (= to C. similis, Townsend, 1917).

Distribution in Mexico; Townsend, 1917.

C. baeri Shannon and Greene.

Alouatta sp. (red howling monkey); British Guiana and Panama; Greene, 1925.

C. beameri Hall.

Neotoma flavidiana osagensis Blair; Kansas; Hall, 1943.

C. buccata Clk.

Citellus tridecemlineatus; United States; Rathron, 1869.

Homo sapiens; Mass.; Bequaert, 1946.

Lepus spp.; Penn. and Kentucky; Brauer, 1863.

Oryctolagus cuniculus domesticus; Georgia; Knipling and Brody, 1940.

Sylvilagus floridanus; Georgia; Knipling and Brody, 1940. Michigan; Haugen, 1942. Oklahoma; Eddy and Emerson, 1940. Recorded in Florida, South Carolina, Penn., Kentucky, New Jersey, Mass., Minn., Nova Scotia; Swenk, 1905.

C. cauterium Clark.

Distribution in United States; Joly, 1846.

C. cayennensis Macquart.

Distribution in Brazil; Patton, 1921.

C. cyanella Jones.

Distribution in Nebraska; Jones, 1906.

C. emasculator Fitsch.

Canis familiaris; Canada (Montreal); French, 1893.

Peromyscus spp.; California; Riley and Howard, 1894.

Sciurus hudsonicus petulans; United States; Seguy, 1924.

S. h. hudsonicus; United States, Bau, 1906.

S. carolinensis leucotis; United States; Seguy, 1924.

Sitomys californicus; California; Riley and Howard, 1895.

Tamias striatus lysteri; Canada; Cameron, 1926. New York, Arkansas, Mass., and Iowa; Fitsch, 1859.

Recorded in New York; Lintner, 1885. In Mexico, Van Der Wulp, 1888. In Illinois, Nova Scotia; Swenk, 1905.

C. ephippium Latr.

Distribution in French Guiana; Patton, 1921.

C. fasciata Swenk.

Tamias spp.; South Carolina, Florida, Miss.; Townsend, 1917. Distribution in Nebraska; Swenk, 1905.

C. fontinella Clark.

Lepus spp.; New Mexico; Townsend, 1892.

Lepus artemisia; United States; Bau, 1906.

Peromyscus leucopus l.; North Carolina, Harkema, 1936.

P. l. noveboracensis; Boston; Johnson, 1930. Maryland, Greene, 1935.

Sylvilagus spp.; New Mexico; Townsend, 1892.

Sylvilagus floridanus mallurus; Smith, 1908.

S. nuttalli n.; New Mexico; Seguy, 1924.

Recorded in British Columbia, Hadwen, 1915. In Illinois; Clark, 1826.

C. funebris.

Loncheres guianae; Trinidad; Bau, 1906.

C. grisea Coquillet.

Mus musculus; Manitoba, Saskatchewan, Alberta, North-West territories, British Columbia; Cameron, 1926.

Citellus tridecemlineatus; Minn.; Washburn, 1905.

C. histrio Coq.

Distribution in Mexico; Coquillet, 1903.

C. horripilum Clk. (= to C. cuniculi, Brauer, 1863).

Canis familiaris; United States; Crawley, 1923.

Felis domesticus; New York; Bequaert, 1946. Missouri and North Carolina; Riley and Howard, 1893.

Lepus spp. and Sylvilagus spp.; Iowa and California; Osborn, 1896. Georgia, Florida, Arizona and Colorado; Virginia and Indiana; Riley and Howard, 1893.

Oryctolagus cuniculus domesticus; United States; Schwartz and Shook, 1933.

Sylvilagus spp.; Georgia; Clark, 1797.

S. floridanus; Virginia and Indiana; Riley and Howard, 1893.

S. floridanus mearnsii; Michigan, Haugen, 1942.

S. palustris; Georgia; Knipling and Brody, 1940.

Recorded in Nova Scotia; Walker, 1849. In Minn.; Lugger, 1896.

C. Jellisoni Curran.

Lepus californicus; Oregon; Jellison, 1942.

C. infulata Lutz.

Distribution in Brazil; Patton, 1921.

C. latifrons Coq. (= to C. americana, Townsend, 1911).

Neotoma fuscipes macrotis; California; Ryckman, 1953.

Recorded in Virginia, South Carolina and Georgia; Townsend, 1917. In California; Coquillet, 1898.

C. lepivora Coq. (= to C. princeps, Townsend, 1917).

Sylvilagus spp.; Wyoming and California; Coquillet, 1898.

Recorded in New Mexico; Johnson, 1902.

C. lepusculi Townsend (= to C. princeps, Townsend, 1917).

Lepus artemisia; New Mexico; Townsend, 1917.

Sylvilagus muttalli n.; New Mexico; Seguy, 1924.

C. megastoma m. (= to C. fontinella, Brauer, 1863).

Lepus spp.; South America; Brauer, 1863.

C. nigricans Lutz.

Distribution in Brazil; Patton, 1921

C. nigrocincta Austen.

Distribution in Brazil; Patton, 1921.

C. nitida Coq. (male is = to C. americana, Townsend, 1917).

Recorded in Virginia, South Carolina and Georgia; Townsend, 1917. In California; Coquillet, 1898. In New Mexico, Swenk, 1905.

C. patagona Guérin (= to C. buccata, Brauer, 1863).

Family Muridae; Patagona, South America; Patton, 1921.

C. peromysci Dalmat.

Peromyscus leucopus noveboracensis; Iowa; Dalmat, 1942.

C. pessoai.

Distribution in Brazil; Guimaraes, 1941.

C. polita Coq. (= to C. americana, Townsend, 1917).

Lepus spp., Virginia, South Carolina, Georgia; Townsend, 1917.

Recorded in Wyoming; Coquillet, 1898.

C. princeps Austen.

Lepus alleni a.; Arizona; Vorhies and Taylor, 1933.

L. californicus c.; Arizona; Ibid.

L. callotis; United States; Bau, 1906. New Mexico; Townsend, 1892.

Sylvilagus floridanus; Arizona, New Mexico, California and Nevada; Townsend, 1917.

Recorded in Mexico; Austen, 1895.

C. ruficrus Austen.

Lepus californicua; Great Basin Area, U. S.; Curran, 1942.

C. rufiventris Macquart

Distribution in Brazil; Patton, 1921.

C. sarcophagoides Lutz.

Distribution in Brazil; Patton, 1921.

C. schmalzi Lutz.

Distribution in Brazil; Patton, 1921.

C. scudderi Townsend

Lepus californicus texianus; Texas; Roberts, 1933.

Suis suis; Maryland, Virginia, Tennessee and Texas; Townsend, 1917.

Sylvilagus floridanus mallurus; Maryland; Townsend, 1917.

C. scuttelaris Brauer (= to C. emasculator, Townsend, 1917).

Citellus spp. and Tamias spp.; N. E. United States; Townsend, 1917.

Recorded in United States; Brauer, 1863.

C. similis Johnson.

Recorded in New Mexico; Johnson, 1903.

C. sterilator Lugger.

Recorded in Minn.; Lugger, 1896.

C. tenebrosa Coq.

Cynomys ludovicianus; Montana; Parker and Wells, 1919.

Neotoma cinerea; Montana; ibid.

Onychomys leucogaster; Montana; ibid.

Recorded in Colorado, Calif., Oregon; Coquillet, 1898.

In South Dakota; Aldrich, 1905.

C. terrisona Walker (= to C. atrox; Aldrich, 1905).

Distribution in Guatemala; Walker, 1849.

C. thomomuris Jellison.

Thomomys borealis; Great Basin area; Jellison, 1949.

C. worontgowi.

Distribution in Brazil; Guimaraes, 1941.

Cuterebra spp.

Citellus tridicemlineatus pallidus; Colorado, Hall, 1910.

Didelphys philander; Brazil; Natterer, 1820 and 1821.

Lepus artemisia; New Mexico; Townsend, 1892.

Lepus palustris; Mexico; Coquerel and Salle, 1862.

Neotoma fallax; Colorado, Hall, 1910.

Neotoma fuscipes macrotis; California; Gander, 1929.

Rattus norvegicus; Canada; Cameron, 1926.

Sciurus aestuans; Brazil, Natterer, 1820 and 1821.

Sciurus aureogaster; Mexico; Coquerel and Salle, 1862.

Sylvilagus floridanus alcer; Oklahoma; Leonard, 1933.

S. nuttalli n; New Mexico; Townsend, 1892.

S. palustris p.; United States; Brauer, 1863.

Thomomys borealis; Wyoming; Leidy, 1857.

STUDIES ON THE LIFE HISTORY AND ECOLOGY OF *CUTEREBRA* SPP. OCCURRING IN MICHIGAN COTTONTAILS WITH SYSTEMATIC STUDIES ON CUTEREBRINE LARVAE FROM OTHER MAMMALS

By

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AN ABSTRACT

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The purpose of this study was (1) to investigate the phases of the life history of Cuterebra sp. as they occur under natural conditions, (2) to elucidate the ecology of the cuterebrid flies in relation to vegetation, soil and host, the Michigan cottontail, Sylvilagus floridanus mearnsii, and (3) to clarify the systematics of cuterebrine larvae collected from certain mammals.

The life history and ecology of Cuterebra species, occuring in Michigan cottontails, were studied in part by means of a two-year live trapping program of rabbits. Field observations on adult cuterebrid flies and rabbits were also carried out. One hundred and seventy rabbits were captured and were screened for Cuterebra larval infections. Infected cottontails were brought into the laboratory and studied for phases of the life cycle.

Records on the duration of third stage larvae in the cottontails related an average of 15 days. Concurrent with the above study, it was found that the air hole diameters of larvae reflected the phases of the third stage larvae while in the host.

Mode of infection studies were done in the field using twenty-two penned cottontail and domestic rabbits in areas having high larval incidences. A positive larval infection was obtained in a penned cottontail which was not exposed to natural vegetation.

The primary site of Cuterebra larval infections in 187 southern Michigan infected cottontails was in the neck. Other anatomical areas recorded were the scrota, cheeks, back and shoulders.

Suggestion of larval migration was observed when two third stage larvae appeared on the right side of the neck of an isolated cottontail which was externally diagnosed negative in that area a week previously. A large tumor appeared first in the area followed by two larval air holes overlying the tumor. No evidences of extensive larval migration were found in sixty-two cottontails which were autopsied throughout the Cuterebra cycle.

The time recorded for the obvious appearance of third stage larvae in cottontails from a negative diagnosis was 22 days.

First, second and third stage larvae were discovered in the cottontails which gave seasonal periodicities in their hosts. The seasonal periodicity of third stage larvae was determined to be between August 1 to 20. First stage larvae were believed to have a seasonal periodicity a month previous to the third stage larvae in the early part of July.

Seasonal incidence of cuterebrid larvae in southern Michigan juvenile cottontails was determined to be between August 1 and 20. For adults, it was found to be between July 20 to 30. In general, the juvenile cottontails had a higher incidence of Cuterebra larvae than adult rabbits.

Also the number of warbles per infected rabbit was found to be the greatest when there was a peak in the incidence of Cuterebra larvae among the cottontails.

Field notes on pupation showed that mature larvae which have recently dropped from their hosts were able to survive 9 to 13 days before pupating. Depths recorded on 27 burrowing larvae offered a range of 1 1/2 to 2 1/2 inches in sandy soils. Field pupation experiments revealed that no two-year cycle existed for the pupae. Also one generation a year was shown to exist in Michigan for Cuterebra, regardless of exposing the pupae to artificial or natural conditions prior to the emergence of the flies. Longevity of adult flies were observed in laboratory pupation jars to range from 1 to 5 days.

Higher numbers of Cuterebra infections were recorded in areas having sandy soils. Also these sandy areas showed greater numbers of rabbit burrows. This situation was believed conducive to good pupation and consequently, successful emergence of adult flies with excellent conditions for larval infections in the rabbit burrows.

Vegetative sites such as dense sumac bushes, willow swales and clover fields were associated with adult cuterebrid flies.

One hundred and twenty-seven cuterebrid larvae, collected from cottontails, cats, dogs, jack rabbits and white-footed mice, were comparatively studied and taxonomic differences

were presented for each instar of individual Cuterebra species studied. The results obtained in the systematic study of larvae were compared with the findings of previous workers. Buttons, peculiar to posterior spiracles of some dipterous larvae, were seen in the cuterebrid examples investigated.

A key is presented which separates the various instars of the individual larval species determined in the systematic study. Species of Cuterebra determined in this study were buccata, fontinella, and horripilum. An unidentified species, found on Nevada jack-rabbits was described and its larval characteristics are presented for comparison with the known cuterebrid species. No pupa-adult fly series were obtained for the identification of the Nevada species. However, they were obtained for the three identified species.

Verification of the three Cuterebra species was accomplished by means of a puparium from which a known identified adult fly emerged. With the morphological characteristics of the puparium it was possible to retrogress to the third instar and subsequently to the first instar of that species.

Larval species found in Felis domestica were C. buccata and horripilum, with a preponderance of the latter species. Cuterebra buccata was recorded from Canis familiaris. Michigan cottontails had both cuterebrid species, with C. horripilum predominating. The only larval species obtained from the white-footed mouse of Michigan was C. fontinella.

A check-list of the Cuterebra species of the western hemisphere is presented with records of their geographic locations and the scientific names of their hosts.

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